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CHROMATOGRAPHIC DETERMINATION OF GLUTAMIC ACID IN SOME PHILIPPINE EDIBLE BEANS AND NUTS

BY LUZ LL. COSME, LOURDES O. LACEBAL, and VIOLETA P. ARIDA

National Institute of Science and Technology, Manila

Local beans and nuts have been evaluated for their essential amino acids(4, 7) but no report on their glutamic acid contents has been published so far. Only peanut, coconut, soybean, pigeon pea and mung bean grown in foreign lands were evaluated for their glutamic acid contents by their investigators,(11, 12, 13) but those varieties grown locally and the rest of our common edible beans and nuts are still undetermined.

L(+)-Glutamic acid $HOOCCH_2CH_2CH(NH_2)COOH$, a non-essential dicarboxylic amino acid occurs naturally in vegetable and animal proteins. It was first isolated from among the products of an acid hydrolysate of gliadin, the alcohol soluble protein of wheat in 1866 by Ritthausen,(15) a German chemist. This chemical gained its commercial importance in 1908 when a Japanese chemist Ikeda(1) discovered that the univalent ion $-OOCCH_2CH_2CH(NH_2)COOH$ of this acid possessed flavor when added to foods. Thus at present(3) the heavy users of the sodium salt of this acid are the manufacturers and producers of canned fish, meats and vegetables. It is sold in tin cans under the following brand names: "Ajinomoto," "Vet-sin," "Mei-Wei-Fen," "Hurron," and "Accent."

Before the war, the United States(3) was a heavy importer of this condiment from Japan and China; however, in 1949 she in turn produced 7.6 million pounds. Later, the Philippines in 1959 imported from the United States, Japan and others 826,133 kilos of this food flavor valued at ₱2,608,088.00

From the medical point of view, some investigators(14) reported the increased mental and physical alertness of patients treated with glutamic acid hydrochloride tablets while others(18) based on their experimentation and clinical observations claimed that this nonessential amino acid may have a genuine facilitating effect on mental functioning in human subjects. Due to the commercial and medical importance of this amino acid, an additional interest was stimulated in knowing how much of it is present in our local beans and nuts.

This paper presents approximate values of the glutamic acid content of 15 local edible beans and nuts made possible by the use of Amberlite ion-exchange resin with modifications. It is intended if it seems feasible to utilize the information so obtained for its future application in the preparation of this amino acid on a commercial scale.

A review of literature on the several methods(5, 6, 8, 9, 10, 17) of separation of glutamic and aspartic acids from protein hydrolysate with the advantages, limitations and inconveniences of each method emphasized, made us prefer the use of ion-exchange resins for our present work. Cannan(5) and Winters(17) reported the efficiency of Amberlites IR-4 and IR-4B, respectively, in their adsorption of dicarboxylic amino acids but we learned from the Resinous Products Division of Rohm and Haas Co. of Philadelphia, Penn., U.S.A., that they are no longer manufacturing these resins. They generously supplied us with Amberlite IR-45 instead for possible application in our work.

EXPERIMENTAL

Amberlite ion-exchange resin IR-45, a weakly basic polystyrene-polyamine type anion exchanger, was examined for its efficiency by employing the first four tests used with Amberlite IR-4 B enumerated in the following paragraph. Results of these tests showed that Amberlite IR-45 adsorbed not only the glutamic and aspartic acids but also some of the neutral amino acids as verified by the ninhydrin test and the paper chromatography of the effluents and eluates obtained. Since the resin failed to serve our purpose, we inquired from other research institutions for any of the recommended resins stated above. Fortunately, the Institute of Hygiene, Manila, had an excess of Amberlite IR-4B (supplied to them also by Rohm & Haas Co.) which was being used in their previous research studies.

For checking the efficiency of Amberlite IR-4B, also a weak base anion exchanger, the following experiments were conducted:

1. A series of columns containing about 5 gms each of fully regenerated Amberlite IR-4B maintained at different pH ranging from pH 5.2 to 5.9 was tested by passing through each column, a 20 cc aliquot of 0.1 per cent aqueous solution of l (+) glutamic acid previously brought to pH 6 with 0.1 N NaOH. Their respective effluents were collected with a flow-rate of 0.5 cc per minute. Qualitative ninhydrin test gave a negative result with the effluent from the resin maintained at pH 5.6 showing the complete adsorption of glutamic acid. The other effluents from resins maintained at pH 5.2, 5.3, 5.4, 5.5, 5.7, 5.8, and 5.9 gave positive reactions with ninhydrin. These results may be attributed to the incomplete adsorption of the glutamic acid.

2. By following exactly the above procedure but using 0.1 per cent aqueous solution of aspartic acid instead of glutamic acid solution, the same results were obtained, that is at pH 5.6 all the aspartic acid were adsorbed. The other effluents mentioned above gave also positive reactions with the ninhydrin test.

3. A solution of a mixture of 2 basic amino acids, lysine monohydrochloride and histidine monohydrochloride, brought to pH 6 with 0.1 N NaOH was passed through Amberlite IR-4B conditioned at pH 5.6. The effluent obtained was found positive to ninhydrin test. When the resin was washed and eluted with 2 per cent sodium carbonate solution, the eluate was found negative to ninhydrin test showing that the basic amino acids were not adsorbed.

4. Similarly, a solution of a mixture of 3 neutral amino acids, cystine, threonine and tyrosine, brought to pH 6 was passed through the resin column, conditioned at pH 5.6. Ninhydrin test applied to both effluent and eluate gave same results as those of the basic amino acids indicating that the neutral amino acids were not adsorbed.

5. Finally, a solution of a mixture of known amounts of glutamic acid, aspartic acid, cystine. threonine, tyrosine, lysine monohydrochloride, and histidine monohydrochloride was adjusted to pH 6 and passed through Amberlite IR-4B column conditioned at pH 5.6. The column was then washed with about 30 cc of distilled water. The effluent was paper chromatographed using normal butanol, acetic acid and water in the ratio of 100 : 18 : 100 as the developing mixture with suitable control. After treatment with 0.1 per cent ninhydrin spray solution, the chromatograms developed showed the presence of both neutral and basic amino acids. The washed resin was eluted with 2 per cent sodium carbonate solution and the eluate was also paper chromatographed in the same way as the effluent. Results showed only the presence of glutamic and aspartic acids in the developed chromatograms.

Interpretation of results of the above tests revealed a convincing evidence that Amberlite IR-4B had a remarkable selectivity for dicarboxylic amino acids and hence applicable to our work.

As for the separation of glutamic acid from aspartic acid, a portion of Foreman's(8) modification of the Ritthausen(15) method was applied and then followed by the Sorensen(16) formol titration method with some modifications.

PROCEDURE

A. Sampling.—The samples used for analysis were obtained mostly from city markets and some from private provincial orchards. The raw edible parts of the nuts without their inner skins as well as the matured edible beans deprived of their pods were used as samples. These were then cut into small pieces with a sharp scalpel, mixed well, and aliquots taken for their moisture, fat and protein analyses (determined by the AOAC method, 1955). The remaining samples were dried at 105°C, ground into fine powder and defatted with ether. These moisture-free and fat-free samples were analyzed for their glutamic acid contents. In the case of the coconut meat, sweet pea and lima bean, their moisture contents were found to be more than 60 per cent, so they were heated at 60°C for about 4 hours prior to their analyses.

B. Hydrolysis.—About 10 gms of the moisture-free, defatted sample was treated with 60 cc of 30 per cent HCl solution in a Florence flask fitted with a water condenser and heated slowly for the first three hours in an oil-bath until frothing has ceased. Heating was continued, allowing the contents to boil for the following 25 hours. After hydrolysis, the flask was disconnected and contents diluted with about 50 cc distilled water, heated again and filtered through prepared Gooch crucible. The residue or the insoluble humin nitrogen collected was washed thoroughly with hot distilled water until washings were neutral to litmus paper. The combined washings were concentrated on a water bath, and later added to the original filtrate of the acid hydrolysate. The excess HCl was removed from the acid hydrolysate by concentration in vacuo. When the hydrolysate became syrupy about 30 cc of distilled water was added repeatedly with continuous heating until distillate was finally slightly acidic. The contents of the distilling flask was transferred to a small Erlenmeyer flask, with the aid of about 50 cc distilled water and decolorized by heating with 1 gm of activated animal charcoal. It was then filtered and the filtrate and washings were made up to 100 cc. Aliquots from this solution were taken for chromatographic adsorption.

C. *Chromatographic adsorption with Amberlite IR-4B.*—The ion exchange resin suited for this determination is Amberlite IR-4B, supplied by Rohm and Haas Co. As per instruction, about 50 gms of the resin was freed from soluble and fine materials by repeated stirring and washing by decantation with distilled water. Alternate treatments successively with 4 per cent hydrochloric acid solution then washing with distilled water followed by 4 per cent sodium carbonate solution and washing with distilled water was done. This cycle of operation was repeated usually 6 to 8 times or until final washing had a pH of 5.6. A 5-gm aliquot of this conditioned slurry was introduced into a vertical glass tube having an internal diameter of 0.8 cm and about 50 cm long. It was provided with a double layer of glass wool at the bottom connected to a smaller glass tube of 1 inch long by a rubber tubing fitted with a clamp. A 10-cc aliquot of the hydrolyzed sample diluted to 20 cc with distilled water was adjusted to pH 6 with N/10 sodium hydroxide. A slight turbidity was usually observed so it was filtered and the precipitate washed with a little distilled water. The filtrate and washings were quantitatively introduced into the prepared column giving 30 minutes allowance for the sample to penetrate the resin before collecting the effluent. The pinchcock was adjusted to a flow rate of 0.5 cc per minute, returning the first cc of effluent back to the column. After the passage of the sample through the resin, about 30 cc distilled water was allowed to run through the column for washnig at the same flow rate. The effluent with the washings was paper chromatographed before being discarded to check if there was any leakage of the glutamic and aspartic acids. (Four of the samples treated in this manner were found to have no leakage and so the rest of the samples were not tested). The washed resin was then eluted with 50 cc of 2 per cent sodium carbonate solution with the same flow rate as that of sample (the last few drops of the eluate should be free from amino acids as revealed by the ninhydrin test).

D. Separation of aspartic acid.—The combined eluate and washings were adjusted to pH 2 with normal sulfuric acid and boiled, adding a little at a time an excess of powdered copper carbonate during the boiling to insure the complete formation of copper glutamate and copper aspartate. It was then filtered while hot and the filtrate was evaporated to dryness. Enough distilled water was used just to dissolve the residue obtained

(the amount usually ranging from 20 to 50 cc) and an equal amount of methanol added. After stirring and setting it aside for 10 minutes, it was again filtered and the residue washed with 10 cc methanol. The collected filtrate and washings were evaporated on a water bath to dryness and the residue obtained was dissolved in 50 cc distilled water, and then heated to 60°C. Afterwards a current of H_2S gas was passed through the solution for 30 minutes. The black sulfide formed was filtered and washed with about 5 cc distilled water. The filtrate with washings was reserved for titration.

E. Sorensen titration method for glutamic acid.—*(1)* *The* clear filtrate was acidified to pH 2 with concentrated HCl and brought back to exactly pH 7 with 0.1 N NaOH. Then 25 cc of 37 per cent formaldehyde solution previously neutralized to pH 7 with 0.1 N NaOH was added to the neutral sample and set aside for 10 minutes after stirring. Finally, using a micro burette, the sample was titrated slowly with 0.1 N NaOH to exactly pH 8.9. The amount of alkali used was recorded. *(2)* Blank Test: Fifty cc of distilled H_2O was treated in the same way as the clear filtrate in *E(1)*. Using only 20 cc of formaldehyde instead of 25 cc the amount of 0.1 N NaOH used was 0.5 cc.

F. Calculations.—Per cent glutamic acid of moisture-free and fat-free sample =

$$\frac{(\text{cc of 0.1 NaOH used} - 0.5 \text{ cc blank}) \times \text{normality} \times 0.147}{\text{Corresponding weight of aliquot taken}} \times 100.$$

Per cent glutamic acid of air-dried sample =

$$\frac{\text{Per cent glutamic acid of moisture-free and fat-free sample} \times [100 \text{ per cent} - (\text{per cent moisture} + \text{per cent fat})]}{100}$$

Per cent protein of moisture-free and fat-free sample =

$$\frac{\text{Per cent protein of air-dried sample}}{100 \text{ per cent} - (\text{per cent moisture} + \text{per cent fat})} \times 100.$$

RESULTS

Portions of procedures previously employed by Cannan, Kibrick, and Sorensen with modifications to suit our purpose have been adopted in this glutamic acid investigation of local beans and nuts. This method, which is found to be time-consuming and not adopted for routine work, has been resorted to because of lack of some of the necessary expensive complicated laboratory

TABLE 1.—*Glutamic acid and protein contents of moisture-free and fat-free beans and nuts. (Arranged according to yield of glutamic acid.)*

English name	Scientific name	Local name	Protein	Glutamic acid	Values reported in literature
			Per cent	*Per cent*	*Per cent*
Peanut	*Arachis hypogaea* Linn.	Mani	46.61	7.93	8.59 [12]
Soybean	*Glycine max* (Linn.) Merr.	Balatong	41.78	6.18	7.64 [12]
Mung bean (green)	*Phaseolus aureus* Roxb.	Munggo (berde)	25.61	5.48	.....
Pigeon pea	*Cajanus cajan* (Linn.) Millsp.	Kadyos	27.18	5.38	.....
Cashew nut	*Anacardium occidentale* Linn.	Kasuy	37.43	5.12	.....
Cowpea (long var.)	*Vigna sinensis* var. *sesquipedalis* Fruw.	Sitaw	22.09	4.76	.....
Pili nut	*Canarium ovatum* Engl.	Pili	61.02	4.60	.....
Mature coconut	*Cocos nucifera* Linn.	Niyog magulang	38.07	4.60	3.90 [11]
Winged bean	*Psophocarpus tetraganolobus* (Linn.) DC	Sigarilyas	43.47	4.56	.....
Hyacinth bean	*Dolichos lablab* Linn.	Bataw	14.54	4.55	.....
Cowpea (short var.)	*Vigna sinensis* (Linn.) Savi	Paayap	21.21	4.30	.....
Rice bean	*Phaseolus calcaratus* Roxb.	Tapilan	20.71	3.64	.....
Snap bean	*Phaseolus vulgaris* Linn.	Abitsuwelas	21.86	3.55	.....
Sweet pea	*Pisum sativum* Linn.	Sitsaro	31.74	3.40	.....
Lima bean	*Phaseolus lunatus* Linn.	Patani	27.64	3.13	.....

TABLE 2.—*Glutamic acid and protein contents of air-dried beans and nuts. (Arranged according to yield of glutamic acid.)*

English name	Local name	Moisture	Fat	Protein (N x 6.25)	Glutamic Acid	Values reported in literature [13]
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Mung bean (green)	Munggo (berde)	11.40	0.80	22.49	4.74	2.81
Pigeon pea	Kadyos	9.37	0.85	24.41	4.72	3.95
Soybean	Balatong	11.29	18.23	29.45†	4.36	7.01
Cowpea (long var.)	Sitaw	12.25	1.29	19.10	4.10	.....
Hyacinth bean	Bataw	11.03	0.76	12.83	4.02	.....
Peanut	Mani	4.67	46.06	22.97††	3.90	5.93
Cowpea (short var.)	Paayap	11.40	1.40	18.50	3.75	.....
Winged bean	Sigarilyas	10.00	14.56	32.80	3.43	.....
Rice bean	Tapilan	11.79	0.57	18.15	3.15	.....
Snap bean	Abitsuwelas	11.23	1.14	19.16	3.11	3.68
Sweet pea*	Sitsaro	10.00	1.49	28.10	2.91	.....
Lima bean*	Patani	5.81	2.77	25.27	2.86	.....
Cashew nut	Kasuy	7.15	44.50	18.10†††	2.47	.....
Mature coconut*	Niyog magulang	11.00	69.09	7.58†††	0.91	.....
Pili nut	Pili	2.77	79.55	10.79†††	0.81	.....

* Fresh sample dried at 60°C for about 4 hours before analysis.
† N x 5.71; †† N x 5.46; ††† N x 5.30.

equipment and chemicals that are known to give accurate results. However, the advantage offered by this method is that only a small amount of sample is needed and that as stated requires only a few simple apparatus and chemicals.

As to the accuracy of the method, an 85 to 90 per cent recovery of glutamic acid from a solution of a mixture of different available amino acids was obtained as verified by paper chromatography. Furthermore, comparison with results of other methods cannot as yet be ascertained as the majority of the samples have not been examined by other investigators. So far, only peanut, soybean, coconut, mung bean and pigeon pea have been analyzed for this particular amino acid by foreign investigators using either the biochemical or microbiological method.

Table 1 shows the approximate glutamic acid and protein contents of 15 local edible beans and nuts computed on a moisture-free and fat-free basis. Samples are arranged according to the yield of glutamic acid. One can see that peanut and soybean have the highest content of glutamic acid while pili nut and peanut lead in the protein content. Glutamic acid contents of peanut and soybean are a little lower than those reported in literature while that of coconut is 0.7 per cent higher.

Table 2 gives the glutamic acid, protein, fat and moisture contents of the air-dried samples. Since the analysis is comparative, those samples containing more than 60 per cent moisture like the sweet pea, lima bean and coconut meat have to be previously dried at 60°C for about 4 hours before analysis. The glutamic acid contents of mung bean and pigeon pea are higher than those reported in literature while those for peanut and soybean are considerably lower. Only the value for snap bean agrees favorably. These variability in results may probably be attributed to the maturity of the sample, geographical location, kind of soil and other as yet unidentified factors.

It is noticeable that samples with high fat content like pili nut, coconut, peanut and cashew greatly reduced their corresponding glutamic acid contents when computed on the air-dried basis while those with less than 1.5 per cent fat were only slightly decreased. Thus mung bean and pigeon pea still lead in Table 2 even if they are only 3rd and 4th, respectively, in Table 1.

Based on our findings, local peanut and soybean are possible

sources of glutamic acid. The rest of the beans and nuts analyzed gave quite low percentages of this amino acid and so a few of them may be utilized as raw materials in the preparation of hydrolyzed proteins, sauces and other food flavorings. Values in the two tables may, however, be a valuable addition to our stock of scientific data on local raw materials. Furthermore, these results may serve as a nutrition guide to children and adolescents in improving their mental efficiencies if experiments along this line, which are still considered tentative, may prove to be effective in the future.

SUMMARY

This paper presents an approximate quantitative determination of glutamic acid in 15 local edible beans and nuts by the use of Amberlite ion exchange resin IR-4B and followed by the Sorensen formol titration method with modifications. The validity of the method has been tested by recovery experiments with pure amino acids and later verified by paper chromatography.

Based on the moisture-free and fat-free samples (Table 1), their corresponding glutamic acid contents varying from 3.4 to 7.9 per cent are given and samples listed according to yield. On the other hand, Table 2 shows the yield of glutamic acid of the same samples but on the air-dried basis, which ranges from 0.18 to 4.74 per cent.

The protein contents of the air-dried as well as the moisture-free and fat-free samples are also shown in the two tables.

Results of investigation show that local peanut and soybean are possible sources of glutamic acid. A few of the samples that follow may be utilized in the preparation of hydrolyzed proteins, sauces and other food flavorings. Values presented may be not only an addition to our scientific data on local materials but also a nutrition guide to children and adolescents in improving their mental efficiencies if experiments along this line may prove to be effective.

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MACRO- AND MICRODETERMINATIONS OF CARBON DIOXIDE COMBINING POWER OF PLASMA OR SERUM

By Felicidad E. Anzaldo, Antonio M. Garcia, and Rogelio N. Relova
National Institute of Science and Technology, Manila

THREE TEXT FIGURES

Metabolic acidosis, which may result from a depletion of body base from an accumulation of acid metabolites, or a combination of the two, occurs as a complication of diabetes mellitus, acute and chronic kidney disease, and diarrheal conditions. Being a clinical entity which occurs not infrequently, its detection is necessary before irreversible chemical changes occur in the body and which may be fatal.

Although the determination of the pH of arterial blood is ultimately necessary to define the acid-base relationship,[1] direct measurements of the blood pH are technically difficult to perform;[2] pH meters are not readily available everywhere in the Philippines. The determination of the capacity of the blood plasma to bind carbon dioxide offers what is probably the most reliable means of detecting acidosis and of measuring its degree.[3]

This report is a comparison of the standard determination of carbon dioxide combining power of plasma and a microprocedure devised by Watts.[4]

MATERIALS AND METHODS

Subjects included in this study were outpatients in the Philippine General Hospital whose condition was not characterized by acid-base disturbance. Of the 123 examined, 22 were males, aged 13 to 60, with a mean age of 34; and 101 females, aged 10 to 68, with a mean age of 29.

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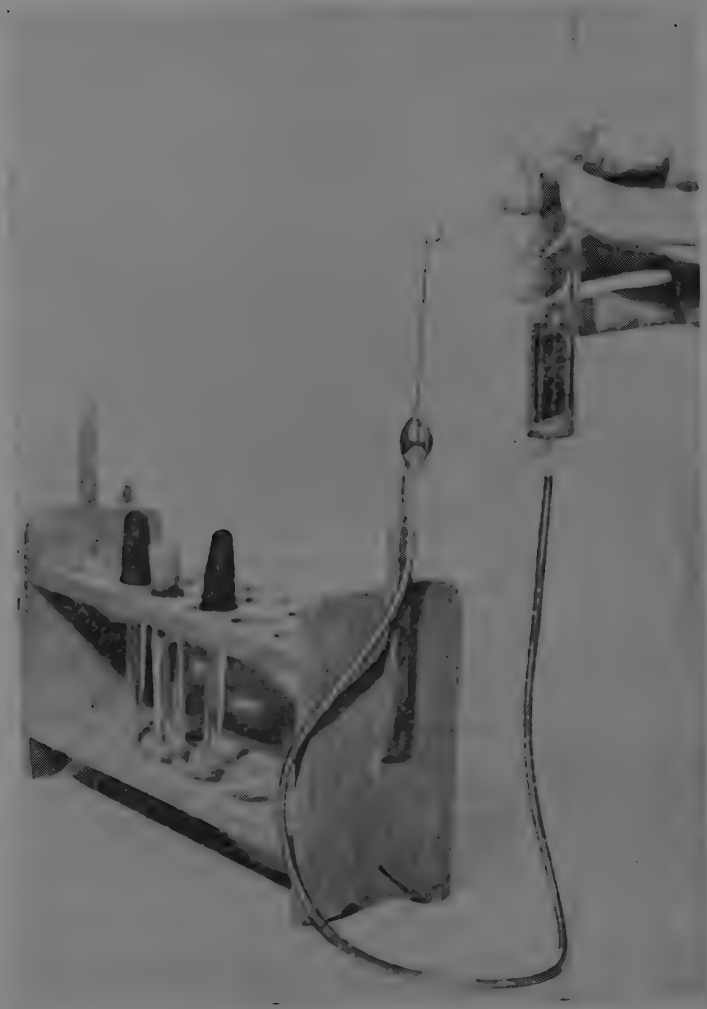

FIG. 1. Volumetric apparatus filled with mercury. Tip of pipette covered with modeling clay.

The subjects need not be fasting. Between four and 5 cc of blood was withdrawn from each subject by venipuncture with the use of a dry syringe. Simultaneously, blood was obtained through a finger prick with the use of a bell-tipped stainless-steel lancet and withdrawn into 120 × 3 mm capillary tubes. One tube for each subject yielded enough serum for one determination. The macrodetermination of carbon dioxide combining power was then made in the manner prescribed by Van Slyke and Cullent as outlnied by Todd, Sanford, and Wells.[5] Microdeterminations were also made on the same day with the use of the method devised by Watts.[5]

The simple volumetric apparatus consisted of a Hellige red blood cell diluting pipette which acted as an evacuation chamber and measuring scale. This was held in a vertical position (Fig. 1), its lower tip connected with a polyethylene tubing to a 10 ml syringe that had been coated with "Lubriseal" to insure airtightness. The apparatus was filled with mercury. As small as 0.01 ml serum or plasma, which had been saturated with alveolar air was introduced, followed by 2.5 volumes of 0.1 N lactic acid, after which evacuation was done several times. CO_2 combining power in volumes per cent was calculated from a given formula.

RESULTS

Table 1 shows the values for the CO_2 combining power of serum or plasma in 123 "normal" individuals. A glance at these values will show the constant smaller values obtained with the micromethod compared with those obtained with the macromethod. Mean value for the macromethod is 53.24 volumes per cent, with a standard deviation of ± 4.49; mean value for the micromethod is 47.28 volumes per cent, with a standard deviation of ± 5.80.

The values obtained with the macromethod as plotted against age, both for males and females, are illustrated in the scatter diagram (Fig. 2). The cœfficient of correlation is 0.07. This is less than the expected standard error of the correlation cœfficient. This statistical evidence shows that the values obtained with the macromethod do not vary with age.

Table 2 shows a comparison of the means of values obtained with the macromethod, in males and females. The deviation of the mean for either males or females from the population

[5] Todd, S. C., et al., loc. cit.

TABLE 1.—*Determination of CO_2 combining power of serum or plasma in 123 subjects.*

No.	Age	Sex	Macromethod	Micromethod
			Volumes *Per cent*	Volumes *Per cent*
1	30	M	59.84	58.73
2	34	F	48.95	47.61
3	53	F	54.93	53.96
4	19	M	50.91	50.79
5	10	F	54.95	52.38
6	52	F	56.98	53.96
7	31	F	50.93	50.79
8	53	F	56.95	55.55
9	45	F	49.94	49.20
10	43	F	56.00	52.38
11	20	M	50.00	47.61
12	20	F	56.00	53.96
13	58	F	51.80	50.79
14	33	F	42.93	41.26
15	17	F	48.78	47.61
16	45	F	46.91	42.85
17	28	F	42.89	41.26
18	34	F	49.96	42.85
19	40	F	50.95	50.79
20	41	F	44.72	36.50
21	34	F	43.94	42.85
22	23	F	50.84	46.03
23	17	F	48.83	47.61
24	24	F	48.85	47.61
25	18	F	51.76	49.20
26	68	F	60.72	60.31
27	32	F	50.74	46.03
28	26	M	52.63	50.79
29	24	F	54.88	53.96
30	49	M	51.87	47.61
31	54	F	43.89	42.85
32	18	F	52.74	50.79
33	29	F	49.77	46.03
34	55	F	48.71	44.44
35	16	M	42.74	39.68
36	54	F	49.61	47.61
37	21	F	47.67	46.03
38	33	F	49.66	47.61
39	29	F	49.66	42.85
40	50	F	52.63	47.61
41	14	F	51.78	47.61
42	23	F	51.78	50.79
43	37	F	53.78	46.03
44	43	F	47.67	44.44
45	50	F	53.62	50.79
46	26	F	68.49	58.73
47	16	M	52.55	47.61
48	26	F	55.54	46.03
49	14	F	51.69	46.03
50	40	M	46.81	46.03
51	49	F	57.69	49.20
52	13	F	45.86	42.85
53	25	F	50.84	47.61
54	24	F	46.81	46.03
55	16	F	57.69	49.20
56	45	M	62.40	53.96
57	15	F	55.42	46.03
58	10	F	54.41	47.61
59	20	F	57.18	49.20
60	30	F	52.42	49.85
61	14	F	56.39	49.20
62	25	F	56.64	49.20
63	20	F	42.44	47.61
64	23	F	55.63	50.79
65	34	F	60.42	58.73
66	15	F	51.26	42.85
67	18	F	54.61	49.20
68	15	F	50.67	42.85
69	30	F	58.59	49.20
70	30	M	58.60	49.20
71	30	F	56.60	47.61
72	22	F	50.61	42.85

TABLE 1.—*Determination of CO_2 combining power of serum or plasma in 123 subjects*—Continued.

No.	Age	Sex	Macromethod	Macromethod
			Volumes *Per cent*	Volumes *Per cent*
73	20	F	58.51	49.20
74	13	F	50.77	41.26
75	39	M	60.73	50.79
76	22	F	52.74	42.85
77	22	F	54.67	46.03
78	18	F	52.55	46.03
79	13	F	49.68	44.44
80	20	F	54.68	46.03
81	48	F	56.55	47.61
82	45	M	56.67	47.61
83	27	F	48.59	42.85
84	18	F	52.58	44.44
85	31	F	54.53	46.03
86	28	F	53.53	47.61
87	47	F	58.38	49.20
88	35	F	52.42	46.03
89	37	F	55.50	47.61
90	47	F	57.48	47.61
91	13	F	56.70	47.61
92	13	M	58.45	50.79
93	40	M	58.32	49.20
94	19	M	50.49	46.03
95	31	F	59.48	50.79
96	18	F	54.40	49.20
97	48	F	58.36	49.20
98	38	M	56.37	47.61
99	28	F	56.34	47.61
100	32	F	48.43	42.85
101	46	F	60.62	50.79
102	23	F	50.55	44.44
103	26	F	55.62	44.44
104	30	F	52.62	49.20
105	47	M	58.53	49.20
106	19	F	50.56	41.26
107	17	F	49.53	46.03
108	12	F	48.52	42.85
109	31	F	52.49	44.44
110	54	M	52.40	47.61
111	56	F	52.40	50.79
112	38	F	50.50	42.85
113	48	F	57.46	47.61
114	26	M	60.42	52.38
115	60	M	60.26	50.79
116	28	F	54.24	46.03
117	20	F	52.40	46.03
118	46	M	59.42	50.79
119	31	M	58.33	50.79
120	55	F	50.48	49.20
121	15	F	56.42	47.61
122	19	F	50.46	46.03
123	25	F	51.46	44.44
Mean			53.24	47.28
Standard deviation			±4.49	±5.80

mean is less than 3 times the standard error of the mean for either males or females. This shows that the values obtained with the macromethod are not related to sex.

Figure 3 presents graphically the relation between the values obtained by the macromethod and those by the micromethod. The cœfficient of correlation is 0.7, which is more than 3 times the expected standard error. This shows that there is a direct

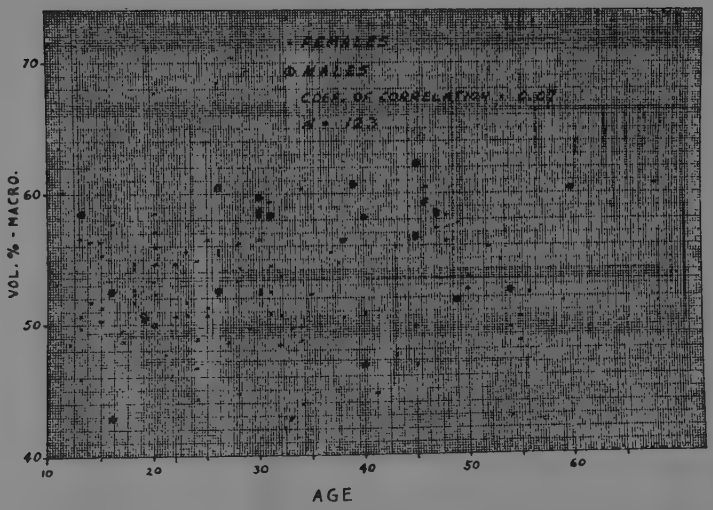

FIG. 2. Scatter of values of macromethod plotted against age.

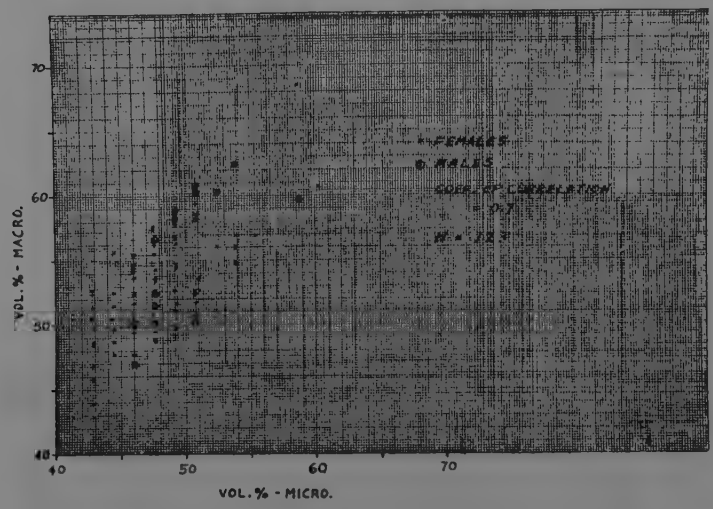

FIG. 3. Relation of values obtained by macro- and micromethod.

TABLE 2.—*Comparison of means of values of males and females (macromethod).*

Sample	Number	Mean	Standard deviation	Standard error of mean
Males	22	55.40	± 5.03	1.07
Females	101	52.76	± 4.30	0.45
All	123	53.24	± 4.49	

relation between the values of the macromethod and those of the micromethod.

Since the values of the micromethod are directly related to the values of the macromethod, it follows that the values of the micromethod are also independent of age as well as of sex.

DISCUSSION

The conventional method of determining the carbon dioxide combining power of plasma as devised by Van Slyke and Cullen requires at least 1 ml of plasma or serum, the withdrawal of blood from a vein, and a gasometric apparatus. It takes about 20 to 30 minutes to make one determination. The method can be done only in laboratories having a Van Slyke apparatus.

The procedure devised by Watts employs the same principle as in the Van Slyke and Cullen method. But the apparatus is simple and inexpensive as it utilizes easily available materials. It takes only a few minutes to make one determination. Since only 0.01 ml serum is needed, blood is obtainable through a finger prick. This makes the microprocedure particularly useful in pediatrics where venipuncture can not always be done with ease.

In this study preliminary trials were done with both macro- and micromethods to determine reproducibility. This was found to be within 5 volumes per cent in both methods.

The direct relationship between values obtained with the micromethod and those obtained with the macromethod and, consequently, the same independence of the micromethod with respect to age and sex, indicates that the micromethod yields results that are just as useful as those obtained with the macromethod.

SUMMARY

Combined macro- and microdeterminations of the carbon dioxide combining power of plasma were performed in 123 indi-

viduals without acid-base disturbance. The microprocedure used was found to be simple and inexpensive, yielding results that were just as useful as those obtained by the conventional macromethod.

ACKNOWLEDGMENT

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CRITICAL STUDY OF THE SAPONIFICATION VALUE OF COCONUT OIL

By D. M. Birosel and A. L. Gonzales

National Institute of Science and Technology, Manila

The difference in the saponification values of quality water-white coconut oil prepared by the authors using two distinct and separate processes was noted by them in their previous work. One oil gave a saponification value of 251.37(1) while another recorded the higher value of 253.89.(2) In a paper(3) published over two decades ago, the senior author and some students reported, among other properties, the saponification value of 255.8 for quality water-white oil prepared by the Lava process,(4) which involves the addition of hydrochloric acid to maintain the pH of the coconut cream between 3 to 5.6. One of the authors has long recognized the fact that when coconut oil is prepared from the coconut cream without the use of chemical, microorganism and heat, the quality water-white oil gives a saponification value of 254.57.(5) Using matured coconuts procured from thirteen different places in the Orient, Allan and Moore(6) reported having obtained saponification equivalents ranging from 214 to 219 and the corresponding saponification values of 262.15 to 255.17, respectively. In the case of the copra oil of commerce the reported saponification value varies from 253 to 263. Copra oil for export supplied to the authors by a large oil mill was found to have a saponification value of 249.86.

The figures cited above show interesting factual variability of the saponification value of coconut oil. Two factors show clearly their effect upon this important chemical property of coconut oil. First, the processing of the oil—the dry or the wet process—affects this important chemical index. The dry process as practised the world over involves the drying of the oleogenous meat or kernel of the nut. The copra oil of commerce is produced by a powerful expeller from the dried meat of matured nuts. Directly opposite this dry process is the newer wet process, from which quality water-white oil is obtained from matured nuts through the production of coconut milk.

The second factor, which also clearly manifests its effect, is the geographical habitat, in which climate, soil and botany of the palm certainly exert an important influence on the nut and its issues. The effect of this factor is very evident on the size of matured nuts which the palms bear in Honolulu and Rio de Janeiro, two cities almost of the same distance from the equator. Coconut palms growing as far north as Hawaii and as far south as Rio de Janeiro bear small nuts; while palms growing in regions near the equator, like Mindanao, Philippines, and Venezuela on the north and Java and Lagoas, Brazil on the south, bear much bigger nuts.

The authors recognize the effect of these two factors on the saponification value, which constitutes a very important chemical index of coconut oil. They do not, however, accept them as total explanation of the interesting phenomenon. Being students of the science called chemistry, they seek for a more comprehensive reason for the factual variability of the saponification value of coconut oil.

The present study includes (*1*) copra oil, (*2*) paring oil, (*3*) Hiller oil, (*4*) I.S.T. quality water-white oil, (*5*) I.S.T. quality rural water-white oil, (*6*) P-M quality water-white oil, (*7*) cold-expressed desiccated coconut meat oil, (*8*) oil from "sapal,"[1] (*9*) from the by-product called "latik,"[2] (*10*) coconut oil with mild pork-lard odor, (*11*) yellow rural oil, (*12*) quality coconut oil from finely comminuted coconut meat preserved in formaldehyde and sodium benzoate, (*13*) coconut oil prepared from cream washed multiple of times with tap water, (*14*) local edible coconut oil marked "X," (*15*) local edible coconut oil marked "Y," and (*16*) three fractions, consisting of lard, middle fraction, and fish-canning oil or salad oil, from coconut oil made by different processes.

Each of these oils are obtained as follows:

(1) Copra oil is produced with the use of the Super-Duo expeller, which operates at fairly high temperatures. After the comminuted copra is free-heated to condition it to right moisture content it is fed to the expeller. The copra oil[3] is press-filtered and stored in big holding tanks where it is allowed to deposit foots of mucilagenous substances.

[1] Comminuted coconut meat residue left after much of the coconut milk has been extracted.

[2] Coagulated proteinous matter produced by boiling coconut milk.

[3] Sample used was furnished by Mr. Jose Vibar of the Scheteleg Oil Factory, San Pablo City.

(2) The paring oil[4] is pressed by the Super-Duo expeller from the dried coconut paring, which consists of the brown coating plus little portions of white coconut meat next to it. Coconut paring is a by-product of desiccated coconut meat factories. Paring oil is yellow-colored; better characterized by low saponification value, which indicates that it consists of glycerol esters of fatty acids of heavier molecular weights.

(3) Hiller oil is produced by the Stanley Hiller oil experimental equipment. This equipment is complete with slicer, rotary dryer, grinder, expeller, a flour mill which produces very rough flour, and a heat producer which requires imported oil. Mature nuts are opened and the meat, which is separated from the shell, is fed to the slicer. From the slicer, the meat is next placed in the rotary dryer where it is thoroughly dried before it goes through the grinder. From the grinder the comminuted meat passes through the expeller, which expresses the oil and allows it to flow into a receiver. The better copra meal is extruded and is finally conveyed to the flour mill which produces from the meal a medium-fine flour.

(4) The I.S.T. quality water-white oil is prepared by eliminating all the other components such as proteins, sugars, salts, starch and etc. of the whole coconut cream excepting the oil with dissolved substances in it. The residue of the cream is finally rendered by gentle heating and continuous stirring to obtain dry oil or a special centrifuge which can deliver dry oil may be used.

(5) The I.S.T. rural quality water-white oil is prepared by careful rendering of whole coconut cream at 100° to 120°C so as not to overcook the proteins which produce the objectionable color and taste. The oil produced by rural housewives turns rancid very quickly, but the I.S.T. rural oil has an exceptional keeping quality. After more than two years, a rural oil sample was found as fresh as when it was first produced. It started with an F.F.A. of 0.03 per cent as oleic acid and after 20 months there was no measurable change.

(6) The Physico-Mechanical(5) or P-M processed quality water-white oil is prepared, like the oils of (*4*) and (*5*), from the cream expressed from finely comminuted meat of ripe coconuts with coconut water added to the "sapal" for second

[4] Same as footnote 3, on opposite page.

pressing to obtain more milk. Under strict temperature control, the liquid state of the oil globules in the cream changes to firm solid and the coconut cream acquires the property of churnability. The solid cream, which is now very much concentrated in fat, is gently melted or just allowed to warm up to room temperature and the cool oil filtered. This oil has exceptional keeping quality surpassing the shelf-life of coconut oils prepared by other processes. It is believed that this oil contains a greater amount of antioxidant than any other coconut oil.

(7) Cold-expressed desiccated coconut meat oil is prepared by cold-pressing in the Carver press laboratory-dried grated meat of ripe coconuts without the brown coating and the little meat next to it. It is dehydrated by absorbing the moisture without heat and then filtered to give out quality water-white oil. A second portion is heated to dehydrate it before filtration to give also quality water-white oil.

(8) Oil may also be obtained from "sapal" or the solid residue of grated and pressed meat of ripe coconuts. The sapal still contains from 10 to 20 per cent of the oil present in the whole meat. It is dried to almost constant weight and oil is extracted with ether. The resultant oil freed from ether is also a quality water-white oil.

(9) "Latik," or the protein by-product from cream, which is drastically heated to obtain brown oil, still contains some oil after filtering the rendered oil. The oil from latik is extracted with ether. It is a dark-brown oil with a nutty burnt odor and flavor when freed from ether and filtered.

(10) Oil with a mild pork odor is encountered whenever the cream has not thoroughly been washed of proteins before rendering it to get the oil. The oil is yellowish. By careful rendering to avoid the browning of the residual protein, the yellowing of the oil and the production of the characteristic mild pork odor are avoided.

(11) Yellow rural oil is obtained when whole cream is rendered and the by-product protein is overcooked but has not turned dark brown. Yellow oil appears before it turns brown. Browning of the oil can be prevented by removing it from the heat.

(12) Quality water-white oil has been produced from comminuted coconut meat which had been kept suspended in wa-

ter and preserved in formaldehyde and sodium benzoate. The sample of comminuted coconut meat used by the senior author came to be produced as a result of a discussion on coconut oil at a symposium of the Philippine Institute of Chemical Engineers. He had made the remark that over 90 per cent efficiency could be obtained by passing the comminuted meat, which was suspended in sufficient amount of water, through the colloidal mill to further subdivide the particles and cause rupture of more oil cells. One of his hearers was so highly impressed with the idea that the latter had a U.S. manufacturer build a comminuter and then sent them coconuts to be comminuted. The processed meat came back preserved in sealed gallon cans, one of which was given the author. He produced from this the quality water-white oil mentioned by using the P-M process.

(13) Quality water-white oil is made from whole coconut cream by the use of tap water in washing it multiple of times. Centrifuge that can deliver dry oil may be used instead of rendering the residue of the washed cream.

(14) and (15) Local edible coconut oils marked "X" and "Y" are bought from the local market. These are produced from copra oil by two widely known manufacturers of edible fats and oils. They use a type of technical processing common the world over. When copra oil has a high F.F.A. (free fatty acid), the oil is washed with alkali solution which, at the same time, gets rid of mucilagenous impurities. It is press-filtered with some kind of filter aid that may also bleach it to some extent at the same time. The partly washed purified oil is steam-treated while admixed with activated black carbon that adsorbs the burnt odor and flavor of the copra oil and does the final bleaching of the oil. The steam carries away the volatile odorous substance that is natural in this oil. Steaming is done for several hours until the oil becomes bland in odor and taste. It is then press-filtered and finally dehydrated and placed in containers. The oil thus purified contains less antioxidant than the original oil from which it is made, and if not reinforced may only have a few months' shelf life. Sooner or later it becomes inedible like the copra oil from which it is derived. This property of edible oil turning inedible is called reversion.

(16) The three fractions of coconut oil are prepared by

a special process[5] which is the direct outcome of an extensive experience in the preparation of various organic substances. This process puts to use especially experience in fractional crystallization to procure the purest alpha- and beta-sativic acids(7) which result from a weak alkaline oxidation of naturally occurring linolic acid in lumbang oil, soya bean oil and other oils, with dilute solution of potassium permanganate.

Allan and Moore(6) showed that the geographical habitat of the coconut palms producing the coconuts of the copra oil of commerce has a marked influence on the saponification equivalent of the oil. They obtained oil from the dried whole meat, from the dried parings consisting of the brown coat and a little portion of meat adjacent to it, and from the dried white-meat portion. The saponification equivalents of the three oils were different; and they were also different from the saponification equivalents of oils from corresponding materials taken from nuts of thirteen different places.

There is no direct measure for the saponification equivalent of an oil. What can be measured directly is the saponification value of the oil, which is the number of milligrams of KOH taken up by 1 gram of the oil. The saponification equivalent of the oil is obtained indirectly by dividing the molecular weight of KOH in milligrams by the number of milligrams of KOH per gram of the oil. The figure obtained is the number of grams of the oil saponified by one gram molecular weight of potassium hydroxide, and it is $\frac{1}{3}$ the molecular weight of the glycerol esters; conversely, the higher the saponification value of the oil, the lower are the molecular weights of the fatty acids composing the glycerol esters.

The authors' experience attests to the fact that the nuts from two provinces adjacent to Manila show no difference in saponification value although the percentages of oil in the nuts vary. Even the oil from much bigger coconuts from Basilan does not show any variation although oil in it is 71 per cent while the Philippine average is only 65 per cent. Working by the P-M (wet) process, 138 cc of oil was obtained from Basi-

[5] The special process to produce 3 coconut fractions, the physico-mechanical process to produce high-quality coconut oil, the stabilization process to produce stable coconut oil, and the production of coconut butter which softens only at a room temperature of 35°C were developed by the senior author during the Japanese occupation of the Philippines when he was not in the Government Service.

lan nuts, 100 cc from Cavite nuts, and from 60 to 80 cc from Laguna nuts, which are much smaller.

The saponification values of differently processed coconut oils are presented in Tables 1 to 3.

TABLE 1.—*The saponification values of the different fractions of P-M quality oil, Hiller oil, paring oil, rural oil and copra oil.*

Kinds of oil	Saponification value
	Average[1]
(a) *P-M Quality oil*:	
Quality oil (cold processed)	254.42
Quality oil (hot processed)	252.89
Stable quality oil	251.37
Lard of quality oil	249.85
Bland coco-lard	247.88
Middle fraction of quality oil	255.65
Bland middle fraction of quality oil	253.22
Liquid fraction of quality oil	258.53
Bland liquid fraction of quality oil	256.87
(b) *Hiller oil*:	
Hiller oil	249.46
Lard from Hiller oil	248.04
Bland lard from Hiller oil	243.70
Middle fraction from Hiller oil	250.86
Bland middle fraction from Hiller oil	249.38
Liquid fraction from Hiller oil	253.43
Bland liquid fraction from Hiller oil	252.32
(c) *Paring oil*:	
Paring oil	236.19
Bland paring oil	233.26
Lard of paring oil	231.18
Bland lard from paring oil	229.61
Middle fraction of paring oil	233.06
Bland middle fraction of paring oil	231.99
Liquid fraction of paring oil	233.62
Bland liquid fraction of paring oil	231.57
(d) *Rural oil*:	
Rural oil	253.89
Bland rural oil	251.73
Lard from rural oil	247.32
Bland lard from rural oil	245.83
Middle fraction from rural oil	253.28
Bland middle fraction from rural oil	251.79
Liquid fraction from rural oil	256.25
Bland liquid fraction from rural oil	254.77
(e) *Copra oil*:	
Copra oil	249.86
Bland copra oil	248.32
Lard from copra oil	241.40
Bland lard from copra oil	240.005
Middle fraction from copra oil	249.87
Bland middle fraction from copra oil	248.65
Liquid fraction from copra oil	251.02
Bland liquid fraction from copra oil	250.16
Stable copra oil	248.15

[1] Average is from 3 different determinations.

TABLE 2.—*Comparison between the saponification values and the saponification equivalent of different kinds of coconut oil.*

Kinds of oil	Saponification value	Saponification equivalent
	average[1]	average[1]
Oil from finely comminuted coconut meat (prepared in the USA and preserved in HCHO and sodium benzoate)	254.71	220.25
Oil from "sapal"	248.88	225.41
Bland "sapal" oil	247.10	227.0
Oil from "latik"	247.31	226.84
Bland "latik" oil	245.67	228.3
Coconut oil with mild pork odor	249.88	224.5
Bland quality coco oil (tap-water processed)	248.49	225.76
Rural oil (yellow)	251.25	223.57
Quality oil from laboratory desiccated coconut, cold processed and filtered cold	250.99*	223.57
Quality oil from laboratory desiccated coconut (cold pressed) oil heated and filtered hot	250.72	223.75
IST quality oil	251.37	223.59
Hiller oil	249.46*	224.88
Copra oil	249.86*	224.53

[1] Average of two determinations.

* Raw Hiller oil, which is a quality oil, and the raw copra oil of commerce are both derived from the dried meat of coconuts. As indicated, their saponification values are 249.46 and 249.86, respectively. The saponification value of the laboratory-prepared desiccated coconut raw oil is 250.99. The latter oil is cold-pressed; and it is presumed that the low temperature used insures its higher saponification value which, nevertheless, is not distinctively much higher than the saponification values of the former. West and Cruz (Philip. Jour. Sci. 41 (1930) 51-58), however, had prepared quality oil by cold pressing desiccated coconut meat and obtained a saponification value of 261, which is considered too high.

The possibilities for error are great when an analyst standardizes his acid with the use of methyl orange as indicator. Even when the neutral point of this indicator has been reached (its pH being between 3 and 4), the solution is still too acidic. The saponification value of several determinations is always higher by a little over 1.53. The high saponification value of an oil from dried coconut meat cannot be reconciled with facts elucidated by the present study.

TABLE 3.—*Comparison between the saponification values of purified and raw coconut oils.*

Kinds of oil	Saponification values	
	Purified	Raw
Bland copra oil	248.13	249.85
Stable copra oil	248.16	249.85
Bland Hiller oil	248.04	249.46
Reblanded edible coco oil "X"	248.09	249.52
Reblanded edible coco oil "Y"	247.94	248.69
Bland cold-pressed desiccated coconut meat oil	248.01	250.99
P-M quality oil washed a multiple of times with distilled water	248.06	254.42
IST bland quality oil	248.10	251.37
Bland quality oil from cream washed a multiple of times with distilled water	248.10 248.02	252.26
Bland rural oil washed a multiple of times with tap water	248.05 248.07	253.89 .....
Average of saponification values	248.06	.....
Corresponding saponification equivalent	226.15	.....

DISCUSSION

Tables 1 to 3 show marked differences in saponification values of the raw oil and its bland counterpart, as well as those of the raw fractions and their bland counterparts, in all the oils prepared by different processes. It is then very

obvious that a definite substance which influences the saponification value of coconut oil is removed when the raw oil is washed with either salt solution, tap water or distilled water. Likewise, when the whole coconut cream or dewatered cream is washed, say twenty times with twice the volume of salt solution, tap water or distilled water, the purified oil gives a saponification value always lower than the saponification value of the raw oil which is obtained from the cream either by the physico-mechanical (P-M) process or by the I.S.T. rural oil process, which is rendering by controlled heating. Quality oil produced by washing coconut cream a multiple of times with twice the volume of salt solution gives a saponification value of 251.37 while quality rural oil gives 253.89 and P-M quality oil gives 254.42.

An oil that is made bland lacks the natural odor and taste of raw oil. In using the term "bland," the authors do not mean that the substance that affects saponification value is the substance that gives the oil its natural odor and taste. The classical way of removing the odor and the taste of the oil is by the use of steam to make the oil bland. Steaming for several hours deprives the oil of its natural odor and taste. The substance that affects the saponification value is not, like the glycerol esters, affected by the steaming of the oil. The only step that edible-fat-and-oil industries can take to decrease this substance is to wash the raw oil with dilute alkali to remove the free fatty acids of copra oil. This substance, which increases the saponification value of all raw coconut oils, will be dealt with in another paper.

The behavior of Hiller oil[5] and copra oil of commerce is quite significant. Both oils are expressed from the dried meat

[5] Considering the saponification values of paring oil (Table 1), of the ether-extracted oil from the solid residue called "sapal," and also of the ether-extracted oil from the protein by-product of rendered cream, it is logical to infer that we are dealing with the glycerol esters of fatty acids of heavier molecular weight. The oil cells which are hardest to press out are those associated with the oldest portion of the meat. The paring, being the oldest portion of the coconut meat, shows the relation of the age of the nut to the saponification value and the unsaturation of the oil.

The white meat left after the paring has been removed contains an oil which gives very much lower iodine value. It is approximately about half the iodine value of copra oil, which varies from 8 to 9, while paring oil gives over 20, depending on how thick the white meat has been pared with the brown coat. It appears that the oil absorbed by the by-product proteins when cream or milk is rendered to give brown oil consists of glycerol esters

of coconuts by expellers (dry process) at different concomitants of heat. The former operates at lower temperature for the object is to produce quality water-white oil from better-quality dried coconut meat. It is surmised, however, that even if the raw material is as white as desiccated coconut meat, the Super-Duo expeller will still produce brown oil as it operates at a temperature high enough to overcook the protein and produce the brown color and burnt odor and taste. When the P-M quality water-white oil is heated to the same range of temperature, no color and flavor change takes place. The source of the dark-brown color of copra oil and nutty burnt odor and taste is very obvious. Hiller oil is water-white; it has a pleasant sweetish fresh coconut flavor, after it is filtered with infusorial earth as filter aid, and with low free fatty acids; while copra oil is highly colored, ill-smelling, with high F.F.A. Their saponification values are almost the same differing only by 0.035, which may be considered within experimental error. Their bland counterparts are lower by 1.5 and differs from each other by 0.26.

The saponification value of the oil prepared by pressing laboratory desiccated coconut meat with the Carver press at room temperature is 250.99, which is higher than the Hiller oil by 1.45 and higher than the commercial copra oil by 1.14. This difference markedly shows that the temperature of expressing the oil from the dried meat of ripe coconuts has an effect on the extractability of the substance that increases the saponification value of the oil. There is more of it in the oil when the temperature of abstracting the oil from the raw material is at a lower level.

This heat effect is even clearer when one considers the wet process of producing the oil. P-M quality water-white oil prepared from dewatered kneaded cream with the entire absence of heat gives a saponification value of 254.42; but the oil in the layer of protein that remains after the P-M quality oil has been separated from the melted dewatered kneaded cream can be secured free from the proteinous aqueous layer by rendering it with gentle heating. This is also a quality water-

of fatty acids of higher molecular weight. It is the lighter oil that separates by rendering; the heavier oil is held by the brown proteins. The oil left with the copra cake and the oil still with the residue of the cold-pressed desiccated coconut meat may follow the trend of the oil left in the "sapal" after expressing the coconut milk.

white oil having a saponification value of 252.75. The difference is very interesting as these two quality water-white oils come from the same batch of dewatered kneaded cream.

The effect of the heat on the substance that affects the saponification value of coconut oil is further made clear in the case of rural quality water-white oil prepared within the range of 100° to 130°C and yellow rural oil produced by overcooking the protein or overheating at temperatures above 130°C. The former gives a saponification value of 253.89 while the latter gives only 251.25. It is therefore very obvious from these experimental evidences that coconut oil prepared at a lower range of temperature will give a higher saponification value than coconut oil produced at a higher level of temperature. Incidentally it is shown that P-M quality oil has the highest amount of this definite substance that affects the saponification value of coconut oil. Although our present knowledge about this substance is meager and limited, we are conscious of its importance or intrinsic role in the stability, rancidity and reversion property of coconut oil.

The edible coconut oils marked "X" and "Y" (Table 3) have gone through a drastic treatment of purification as followed in edible-fat and oil factories. The change of the saponification value of edible oil "X" to 249.52 is only 0.33 below the saponification value of copra oil from which it has been derived. The decrease may have been caused by the dilute alkali washing made on the raw copra oil to eliminate the free fatty acids. The substance that increases saponification value of coconut oil is still in the edible oil. The decrease of 1.16 in the saponification value of edible oil "Y" from the value of copra oil from which it has been derived means that the alkali treatment of the raw oil is more extensive and drastic.

It is known that edible coconut oil has the propensity of turning to its former bad qualities which is appropriately called "reversion property." Sooner or later edible coconut oil will develop odor and flavor which render it unfit for human consumption. It is premature to associate the substance which increases the saponification value with the cause for reversion, for it is a fact that copra with a moisture content of over 10 per cent is a good breeding ground for microorganisms. These may have elaborated organic substances that act as prooxidant working against a small amount of antioxidant in

the edible oil derived from the copra oil that has already turned rancid. P-M quality oil, however, has been observed to maintain long shelf life over years; and it has been experimentally demonstrated that after six months of shelflife it still shows an induction period of 16 hours at 105°C in which the peroxide value is only a quarter of a point with zero registering at the beginning.

The ten different coconut oils recorded in Table 3 give an average saponification value of 248.06. This may be taken as the saponification value of what may be considered pure coconut oil. The substance that increases the saponification value was exhaustibly eliminated by washing the oil with either salt solution, tap water, or distilled water, and this treatment was continued until the last two consecutive saponification values differed only by a few digits, say 2 or 3, on the second decimal point. The authors do not claim that they have completely eliminated the substance although its effect is already nil. In the case of Hiller oil, bland quality oil from cream, bland rural oil, of whcih two samples are still retained, the reproduceability of saponification values is still obvious.

The implication of the saponification values of the different fractions of the different oils is very evident. The raw fractions always have higher saponification values than their bland counterparts. The substance that affects the saponification has been reduced. In all the oils that have been fractionated, the coco-lard fraction has the lowest saponification value and the liquid fraction has the highest. That of the middle fraction, although in liquid state, lies between the two. The lard fraction then is made up of glycerol esters of fatty acids of higher molecular weight while the liquid fraction is made up of glycerol esters of fatty acids of lower molecular weight. The lard of P-M quality oil in Table 1 gives a saponification equivalent of 224.53, which represents the weight of the oil in grams reacting on one gram molecular weight of KOH. Fats and oils happen to have three equivalents. The molecular weight of the lard then is 673.59. The saponification value of the liquid fraction is 258.53 and the corresponding saponification equivalent is 216.99, which gives 650.97 as the molecular weight of the liquid fraction. The implication of the molecular weight is very obvious.

The column under saponification equivalent in Table 2 shows that the molecular weight of the bland counterpart of

the raw oil is appreciably higher than the molecular weight of the raw oil. The molecular weight of the substance that has been eliminated to make the oil bland must necessarily be lower than the weight of the raw oil. When this substance is eliminated to make bland oil, the unsaturation of the bland oil is always found to be lower than the original oil, as shown by its iodine value. This means that the substance eliminated is unsaturated and that it is oxidizable. These observable facts relating to the substance that has direct effect on saponification give support to our earlier proposition that it plays a direct part in the two big problems of edible fats and oils that have excited the interest of fat-and-oil chemists the world over for a considerable period of time.

SUMMARY

1. There is a definite substance that dissolves in the coconut oil when it is abstracted from the dried meat by the expeller method (dry process) or by the newly developed methods (wet processes) that produce quality water-white oil direct from milk expressed from comminuted fresh meat of ripe coconuts. This substance markedly affects the saponification value of the oil and, significantly, its saponification equivalent.

2. The temperature at which the oil is produced from the raw materials determines how much of this definite substance will be present in the abstracted coconut oil. Oil produced at low temperatures contains more of the substance than the oil prepared at higher temperatures, even with the use of the same process. Wet-processed oil has more of it than the dry-processed.

3. Fundamental cause of the variability of saponification value of an oil has been presented. Interesting subjects of research have been indicated.

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NORMAL VALUES FOR BLOOD CHEMICAL CONSTITUENTS AMONG FILIPINOS*

By Rosa Guevara
University of Santo Tomas, Manila
and
Virginia G. Cabueños
Public Health Chemist, Manila

TEN TEXT FIGURES

Data on the normal values of blood chemical constituents among Filipinos are scanty, and although studies have been made during the last 35 years, the values for only a few constituents have been reported. In 1926 Paras(5) worked on seventeen normal staff members of the Culion Leper Colony and gave the values for nonprotein nitrogen, urea nitrogen, uric acid, creatinine, sugar and the chlorides. At about the same time Navarro and Cruz reported values for uric acid, creatinine and sugar. In 1927 Concepcion and Ocampo(2) analyzed the blood of 48 medical students and also reported the values for nonprotein nitrogen, urea nitrogen, uric acid, creatinine, sugar and chlorides. In 1939 Celis and Cruz[1] determined the values for pH on about 1,000 normal individuals. In 1940, Bona and Concepcion(1) worked on 33 normal students and gave values for total proteins, albumin, globulin and albumin/globulin ratio. In 1952 Samson and Cordero(6) reported values for nonprotein nitrogen and sugar of 102 normal medical students. In 1955 Camara-Besa[2] reported on sodium, potassium and plasma proteins.

Thus far the normal values for only 12 blood chemical constituents have been reported for Filipinos. The authors thought of doing further studies on the blood chemistry of normal Fili-

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First Prize winner of the 1960 Singian-Litton Award for Basic Science by U.S.T. Medical Association, and Second Prize winner of the 1960 Research Award for Basic Science Division by the Manila Medical Society.

[1] Bull. San Juan de Dios Hospital 13 (1939) 427-433.

[2] Acta Med. Phil. 12 (1955), 13 (1956).

pinos using a bigger number of cases and having in view, if possible, all of the blood chemical constituents.

The main aims in this study were (*1*) to establish a range of values that would indicate the percentage of cases falling within this range; (*2*) to determine the values for males and for females; and (*3*) to determine the values in the different age groups.

Normal values are usually reported as a single range which does not mention percentage of the cases falling within that range. In 1951 Wotton and King(9) rpeorted normal values of blood obtained from different hospitals in London and he gave ranges which included 1 per cent, 10 per cent, for the lower limit and 10 per cent, 1 per cent for the upper limit so that each range gives us the percentage it includes. In this study the writers have established a range of the normal values with the percentage of the cases that falls within this range.

In literature only cholesterol has been mentioned as the object of studies with reference to sex and age. With growing interest in geriatrics because of the increasing number in the elderly portion of the population, comparative blood analysis in the different age groups may be of value in studies about the physiology of aging.

CASES STUDIED

Blood samples were taken from 1,314 normal adults, 638 of whom were males and 676 were females, with the ages ranging from 15 to 68 years. Many of them were college students, some were teachers, and others were employees and laborers of various government bureaus. All were known to be free from any systematic disease as determined by physical examinations, including chest x-rays; laboratory examinations of the urine and stool; and serological tests.

Sampling of blood was done uniformly, i.e., venous blood was taken always before breakfast since it is generally accepted that digestion has some influence on the chemical constituents of the blood.[3] Analysis was completed on the same day the blood specimens were taken because long standing favors bacterial decomposition and auto-enzymatic action in them.

Normal figures established by other authors were obtained under the above mentioned conditions, so adherence to these standards, were followed for accurate comparisons.

[3] Kolmer, Clinical Diagnosis by Laboratory Methods.

METHODS USED

The following methods, which were modified by Leitz,[4] were used for the analyses of the different chemical constituents:

1. Folin and Wu: For nonprotein nitrogen, uric acid, creatinine, and sugar.
2. Karr's Direct Nesslerization: For urea nitrogen.
3. Greenberg: For total proteins, albumin, globulin, and albumin globulin ratio.
4. Bloor: For cholesterol and total lipids.
5. Beckman Flame Photometer: For sodium and potassium.
6. Sendroy: For chlorides.
7. Clark: For calcium.
8. Malloy and Evelyn: For total bilirubin.
9. Muellengracht: For icterus index.
10. Somogyi: For amylase.
11. Comfort and Osterberg: For lipase.
12. Marciogen: For thymol turbidity.
13. Bodansky: For alkaline phosphatase.
14. Gertman and Gutman: For acid phosphatase.
15. Fiske and Subbarrow: For phosphates.
16. Exten and Schatiner: For CO_2 combining power.
17. Sigma Tech. Bull: For S G O T and S G P T.
18. pH Meter: For pH.

The above-mentioned methods were adapted to the Rouy photometer, the colorimeter used in reading the results.

RESULTS AND DISCUSSIONS

The results of the analyses were organized by a mathematician who helped the authors determine the range of values for each chemical constituent. This was done by establishing a frequency distribution. The descriptive measures used here are the mean, median and mode, all of which are measures of central tendency.[5] When the mean, median, and mode are the same or nearly so, a normal distribution is indicated.

To establish the range, percentile[6] is used. This divides the total number of cases into 100 equal groups. Thus 10 percentile is the point below which 10 per cent of the cases lie and above which 90 per cent of the cases will occur; and 90 percentile is the point below which are 90 per cent of the cases and above which are only 10 per cent of the cases.

[4] Handbook of Rouy Photometer.
[5] Guilford, Fundamental Statistics in Education.
[6] Mills, Statistical Methods.

TABLE 1.—*Values of chemical constituents of blood of 1,314 normal Filipinos.*

Constituent	Minimum	Maximum	Mean	Median	Mode	10 Percentile	90 Percentile
Nonprotein nitrogen mgs/100 cc blood	21.0	41.0	32.1	31.7	30.8	26.3	38.3
Urea nitrogen mgs/100 cc blood	8.1	17.8	12.6	12.4	Bimod.	9.9	14.6
Uric acid mgs/100 cc blood	2.0	4.2	3.25	3.2	3.1	2.6	4.0
Creatinine mgs/100 cc blood	0.9	2.0	1.38	1.38	1.36	1.09	1.68
Total proteins gms/100 cc serum	4.9	8.7	6.91	6.81	6.58	5.09	7.97
Albumin gms/100 cc serum	3.0	5.5	4.21	4.21	4.25	3.53	4.91
Globulin gms/100 cc serum	1.18	4.0	2.7	2.7	Bimod.	2.06	3.33
Albumin/Globulin ratio	0.1:1	2.6:1	1.6:1	1.5:1	1.4:1	1.2:1	2.1:1
Sugar mgs/100 cc blood	78.0	120.0	95.0	95.0	93.0	83.0	107.0
Cholesterol mgs/100 cc blood	130.0	251.0	184.0	183.0	182.0	152.0	219.0
Sodium mEq/L	121.0	158.0	140.0	139.5	140.0	127.0	149.3
Potassium mEq/L	2.5	4.8	3.45	3.0	3.3	2.7	4.3
Chlorides as NaCl mgs/100 cc serum	514.0	726.0	631.0	642.0	650.0	568.0	691.0
Calcium mgs/100 cc serum	7.0	12.0	9.7	9.9	Bimod.	8.1	12.0
Total Bilirubin mgs/100 cc serum	0.1	0.55	0.27	0.26	0.25	0.13	0.36
Icterus Index units	2.3	6.8	5.1	5.5	5.9	3.6	6.2
Amylase units	60.0	180.0	96.0	93.0	Bimod.	78.0	135.0
Lipase cc of N/20 NaOH	0.2	1.5	0.55	0.55	Bimod.	0.21	0.92
Thymol turbidity units	0.2	4.7	2.79	2.9	Bimod.	0.93	3.8
Alkaline phosphatase units	0.3	5.4	2.98	3.08	3.07	1.3	4.7
Acid phosphatase units	0.4	3.9	1.65	1.45	Bimod.	0.6	3.2
Phosphates mgs/100 cc serum	1.3	4.0	2.96	2.93	2.75	2.23	3.97
CO_2 combining power vols. per cent	42.0	78.0	60.1	60.0	Bimod.	47.7	74.2
SGOT units	8.0	40.0	20.0	19.0	21.0	9.0	34.0
SGPT units	5.0	33.0	17.0	15.0	Bimod.	8.0	26.0
Total lipids mgs/100 cc	350.0	600.0	473.0	500.0	502.0	399.0	551.0
pH	7.25	7.8	7.46	7.47	7.52	7.36	7.59

Table 1 shows the values of the different blood constituents of 1,314 normal cases studied. It will be noted here that the three measures of average—mean, median and mode values—for the various constituents do, in most cases, coincide with one another. This is indicative of a fairly good distribution and representative sampling of the cases.

COMPARISON OF VALUES FOR MALES AND FEMALES

The percentage of cases and the values for males and females are given in Table 2. Graphic presentations are also shown in Figs. 1 to 4 and differences noted.

The values are higher in males for nonprotein nitrogen, urea nitrogen, uric acid, creatinine, chlorides, sodium, bilirubin, icterus index, amylase, lipase, alkaline, phosphatase, acid phosphatase, phosphates, CO_2 combining power, SGOT and SGPT. They are a little higher in females for total proteins, albumin, globulin, thymol turbidity, calcium, and total lipids. For potassium, sugar, cholesterol, and pH, they are about the same for males as for females.

Little is said of the differences in the values for males and females in literature. Among the few blood chemical constituents mentioned is cholesterol. British investigators, like Hobson, Jordan, and Roseman,(3) have reported higher values for women (310 mgs per cent) than for men (268 mgs per cent) of the same age. "For both young and old, mean values in women are significantly higher than in men."

VALUES IN THE DIFFERENT AGE GROUPS

The ages in all our cases range from 15 to 68 years. We have divided them into three age groups: Group 1, from 15 to 29 years; Group II, from 30 to 44 years; and Group III, from 45 to 68 years. The values for each age group are shown in Table 2 and Figures 6 to 8. It will be noted that nonprotein nitrogen, urea nitrogen, uric acid, creatinine, cholesterol, total lipids, sodium, amylase, alkaline phosphatase, SGOT and SGPT are higher in the older age group. Calcium, potassium, chlorides, CO_2 combining power and thymol turbidity have higher values in the younger age group; total proteins, sugar icterus index, bilirubin, lipase, acid phosphatase, phosphates, and pH are about the same in all age groups.

As to the differences of values in the young and old, again cholesterol has been the subject of investigation by various

TABLE 2.—*Comparison of values of chemical constituents of blood of Filipinos (male and female).*

Constituent	No. of cases	Sex	Minimum	Maximum	Mean	10 per-centile	90 per-centile
	Per cent						
Nonprotein nitrogen mgs/100 cc blood		M	23.5	41.0	34.5	29.0	39.7
		F	21.0	40.0	30.8	24.6	35.8
Urea nitrogen mgs/100 cc blood		M	8.1	17.8	13.0	10.6	15.8
		F	8.2	17.6	12.2	9.6	14.6
Uric acid mgs/100 cc blood		M	2.2	4.2	3.6	2.8	4.1
		F	2.0	4.0	3.1	2.5	3.9
Creatinine mgs/100 cc blood	110=33*	M	0.97	2.0	1.54	1.2	1.9
		F	0.9	2.0	1.3	1.1	1.5
Total proteins gms/100 cc blood		M	5.3	8.7	6.7	5.7	7.8
		F	5.0	8.5	7.0	6.1	8.1
Albumin gms/100 cc serum		M	3.0	5.5	4.18	3.4	4.8
		F	3.0	5.5	4.3	3.6	5.1
Globulin gms/100 cc serum	224=67**	M	1.6	3.7	2.6	2.0	3.2
		F	1.2	4.0	2.9	2.1	3.4
Albumin/Globulin ratio		M	1.4:1	2.5:1	1.6:1	1.2:1	2.1:1
		F	1.0:1	2.5:1	1.5:1	1.2:1	2.1:1
Sugar mgs/100 cc blood		M	78.0	119.0	96.0	82.0	109.0
		F	80.0	120.0	95.5	83.0	104.0
Cholesterol mgs/100 cc blood		M	135.0	251.0	184.0	151.0	224.0
		F	130.0	242.0	184.0	152.0	217.0
Sodium mEq/L	109=54*	M	121.0	157.0	141.0	128.0	150.0
		F	121.0	158.0	138.5	127.0	149.0
Potassium mEq/L	91=46**	M	2.5	4.76	3.5	2.9	4.0
		F	2.6	4.65	3.5	2.9	4.2
Chlorides as NaCl gms/100 cc serum		M	529.0	726.0	633.0	569.0	691.0
		F	514.0	726.0	626.0	582.0	692.0
Calcium gms/100 cc serum	56=56*	M	7.0	12.0	9.5	8.0	11.7
		F	8.0	12.0	10.6	8.2	12.0
Total bilirubin gms/100 cc serum		M	0.1	0.55	0.29	0.14	0.42
		F	0.1	0.50	0.26	0.13	0.35
Icterus index units	46=45**	M	2.3	6.8	5.1	3.7	6.3
		F	2.3	6.2	5.0	3.6	6.1
Amylase units		M	72.0	180.0	99.0	79.0	147.0
		F	60.0	166.0	93.0	76.0	124.0
Lipase cc of N/20 NaOH	60=57* 45=43**	M	0.2	1.5	0.7	0.2	1.0
		F	0.2	1.5	0.5	0.2	0.9
Thymol turbidity units		M	0.2	4.7	2.5	0.9	4.4
		F	0.4	4.7	3.0	1.4	3.1
Alkaline phosphatase units	71=54*	M	0.3	5.4	3.0	1.1	4.7
		F	1.3	3.1	2.7	1.9	3.7
Acid phosphatase units	61=46**	M	0.5	3.9	1.3	0.7	3.2
		F	0.4	3.6	1.5	0.5	2.8
Phosphates mgs/100 cc serum		M	2.0	4.0	3.1	2.2	3.9
		F	1.3	3.8	2.9	2.2	3.8
CO_2 Combining power vols. per cent		M	42.0	78.0	62.0	47.0	74.0
		F	48.0	76.0	58.0	47.0	71.0
S G O T units	51=51*	M	8.0	39.0	21.0	10.0	32.0
		F	8.0	40.0	20.0	9.0	35.0
S G P T units	49=49**	M	7.0	33.0	17.0	9.0	26.0
		F	5.0	33.0	15.0	6.0	25.0
Total lipids mgs/100 cc	70=58* 50=42**	M	350.0	600.0	454.0	397.0	514.0
		F	400.0	600.0	493.0	404.0	552.0
pH	111=50* 110=50**	M	7.25	7.8	7.47	7.36	7.59
		F	7.3	7.8	7.46	7.35	7.58

* Male; ** Female

TABLE 3.—*Mean values of chemical constituents of blood of Filipinos in different age groups.*

Constituent	No. of cases	15 to 68 years old	I 15 to 29 years old	II 30 to 44 years old	III 45 to 68 years old
	Per cent				
Nonprotein nitrogen mgs/100 cc blood		32.1	31.3	33.2	33.7
Urea nitrogen mgs/100 cc blood		12.6	12.3	12.4	13.1
Uric acid mgs/100 cc blood	I—205–61	3.25	3.13	3.2	3.3
Creatinine mgs/100 cc blood	II— 67–20	1.38	1.35	1.4	1.48
Total serum proteins gms/100 cc	III— 62–19	6.91	6.92	6.72	6.92
Sugar mgs/100 cc blood		95.3	96.0	94.4	96.8
Cholesterol mgs/100 cc blood		183.5	181.1	191.0	192.8
Sodium mEq/L		139.5	138.0	138.0	142.0
Potassium mEq/L	I— 74–37 II— 64–32 III— 62–31	3.4	3.5	3.5	3.3
Chlorides as NaCl gms/100 cc serum		630.5	633.3	632.0	614.0
Calcium gms/100 cc serum	I— 41–40	9.7	10.0	9.5	9.4
Total bilirubin gms/100 cc serum	II— 40–40	0.27	0.25	0.28	0.26
Icterus index units	III— 20–20	5.07	5.05	5.17	5.0
Amylase units		96.3	90.5	101.1	99.4
Lipase cc of N/20 NaOH	I— 31–30 II— 39–36 III— 35–34	0.55	0.54	0.55	0.55
Thymol turbidity units		2.79	3.29	2.34	2.64
Alkaline phosphatase units	I— 52–40	2.98	2.98	2.7	3.4
Acid phosphatase units	II— 40–30	1.65	1.63	1.72	1.6
Phosphates gms/100 cc serum	III— 40–30	2.96	2.97	3.1	2.9
CO_2 Combining power vols. per cent		60.0	62.5	60.5	59.5
S G O T units	I— 24–24	20.0	18.0	20.0	22.0
S G P T units	II— 36–36 III— 40–40	17.0	14.0	16.0	17.0
Total lipids mgs/100 cc	I— 38–32 II— 36–39 III— 46–38	473.0	461.0	473.0	505.0
pH	I— 45–20 II— 76–35 III—100–45	7.47	7.47	7.48	7.47

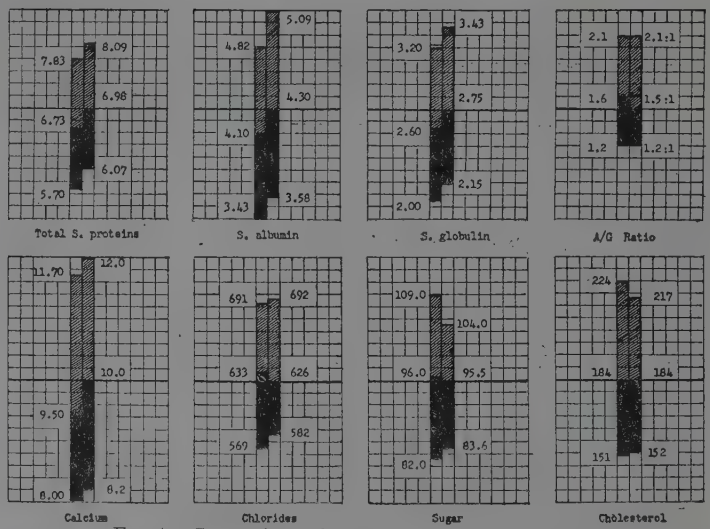

FIG. 1. Comparison of values for male and female.

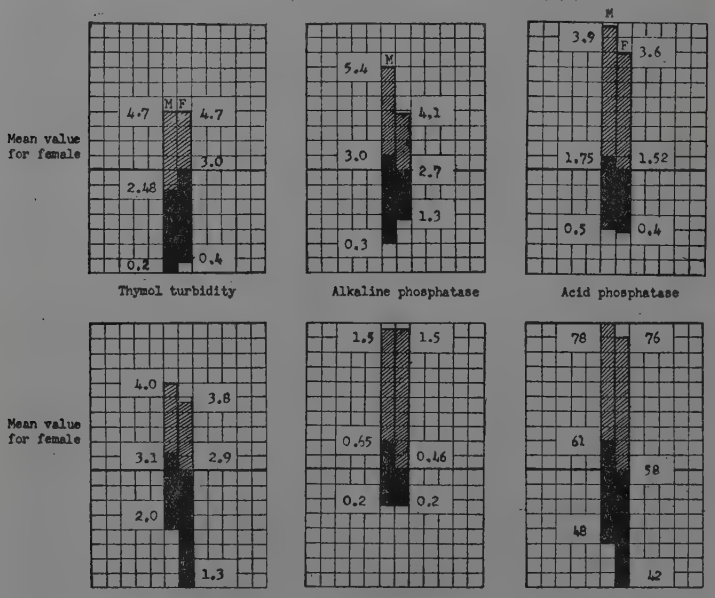

FIG. 2. Comparison of values for male and female.

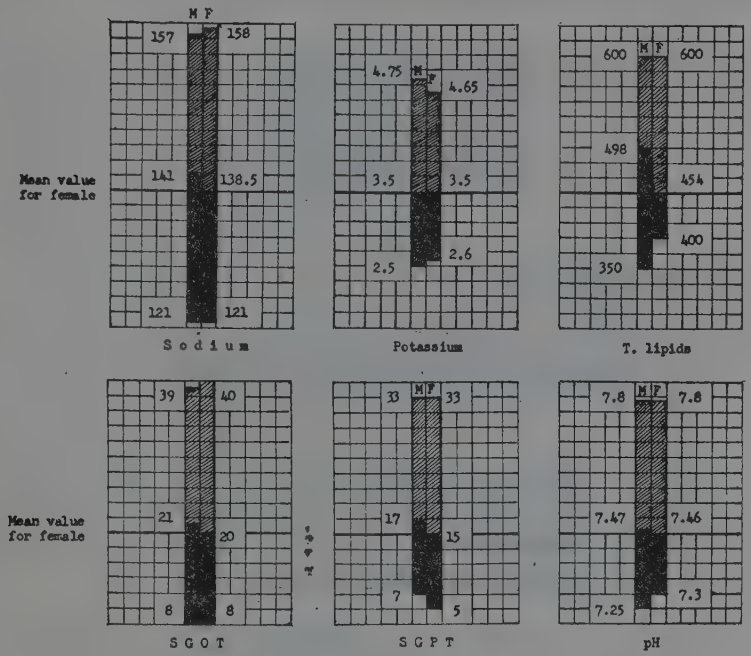

FIG. 3. Comparison of values for male and female.

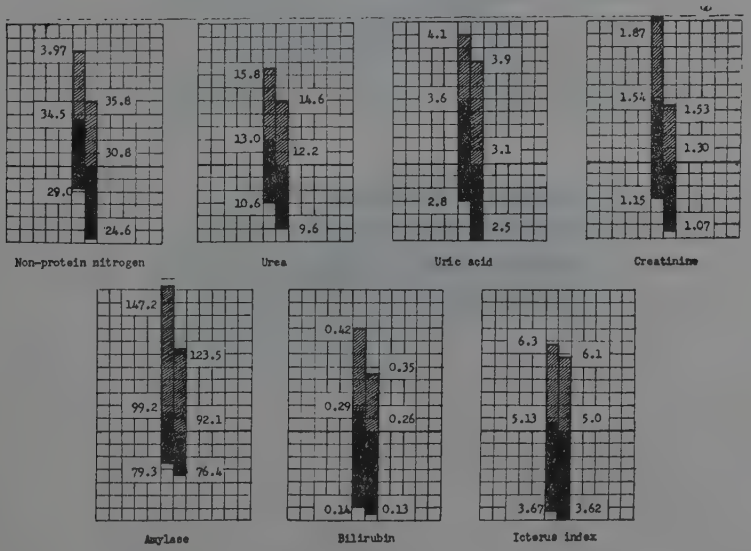

FIG. 4. Comparison of values for male and female.

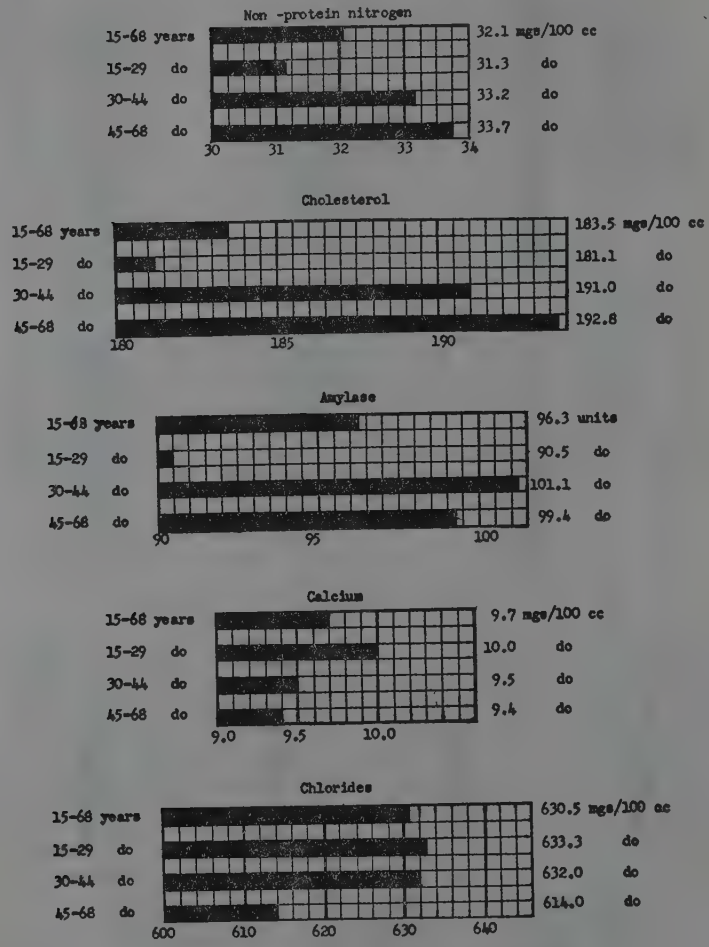

FIG. 5. Mean values in different age groups.

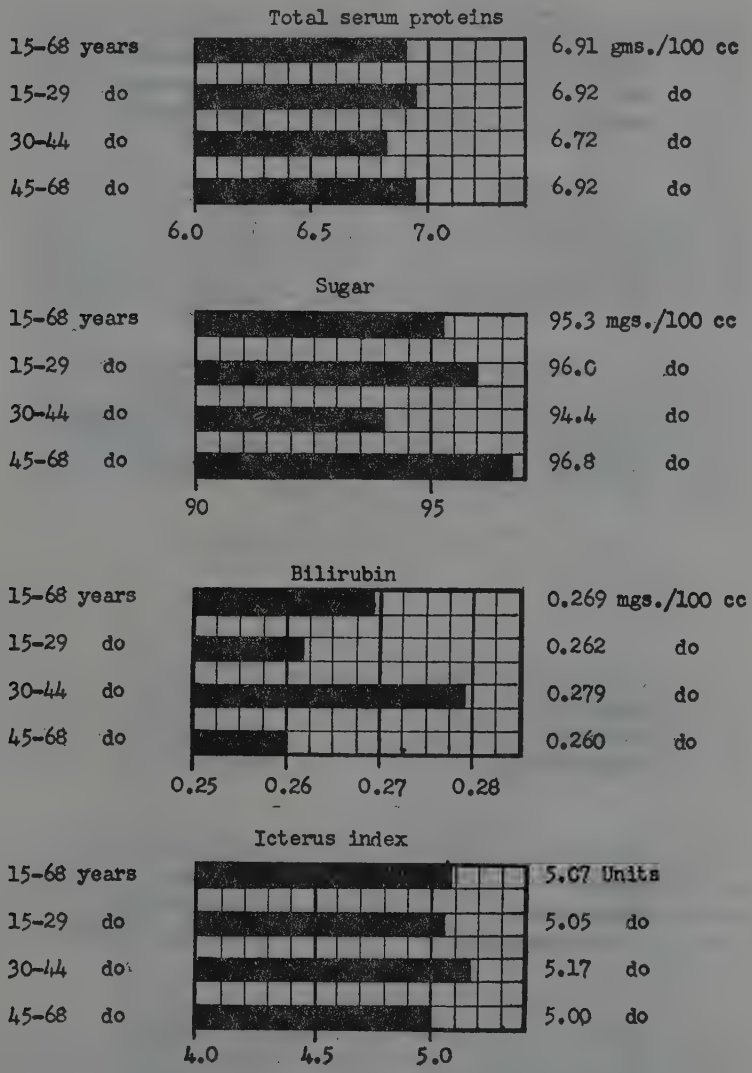

FIG. 6. Mean values in different age groups.

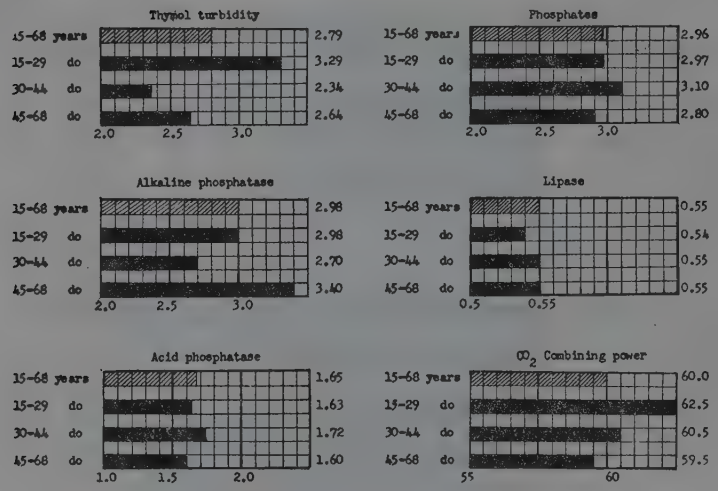

FIG. 7. Mean values in different age groups.

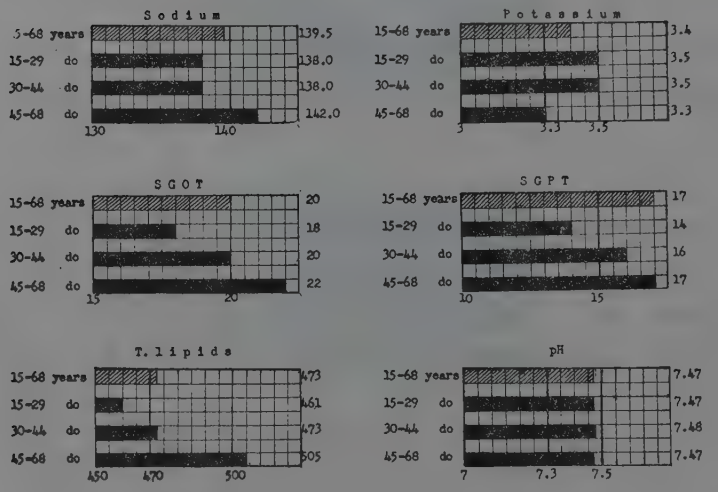

FIG. 8. Mean values in different age groups.

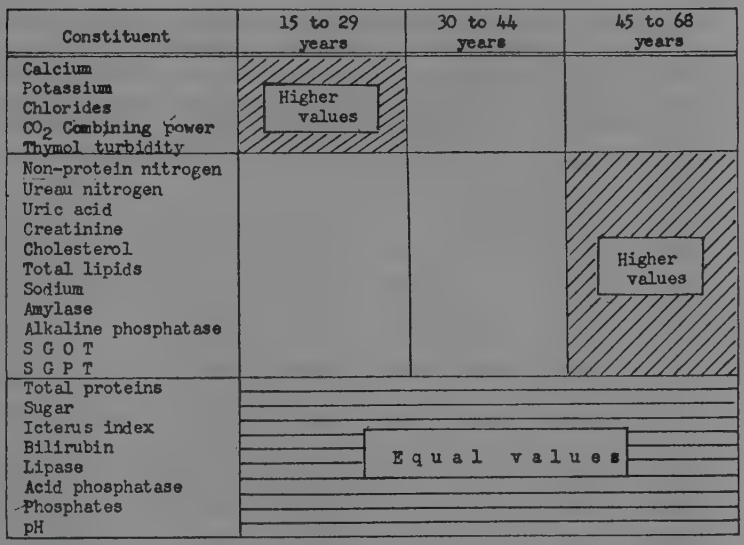

FIG. 9. Summary of comparison of values as to age.

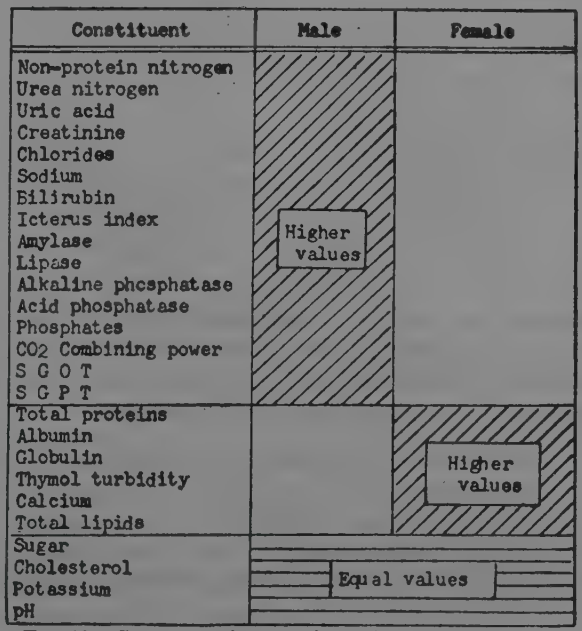

FIG. 10. Summary of comparison of values as to sex.

TABLE 4.—*Comparison of values of chemical constituents of blood of Filipinos obtained by different authors.*

Constituent	Paras (1926) 17 cases	Navarro and Cruz (1926) 50 cases	Concepcion, Ocampo, and Bona (1927-1940) 48 cases	Samson and Cordero (1952) 102 cases	Camara-Besa (1952) 100 cases	This series (1959-1960) 534 cases
Nonprotein nitrogen mgs/100 cc blood	28.3*	—	29.1*	35.4*	—	32.1 ††
Urea nitrogen mgs/100 cc blood	14.3*	—	14.5*	—	—	12.6 ††
Uric acid mgs/100 cc blood	3.5*	3.85*	3.7*	—	—	3.25††
Creatinine mgs/100 cc blood	1.2*	1.65*	1.5*	—	—	1.38††
Sugar mgs/100 cc blood	96.7*	89.5**	107.2*	100.5*	—	95.3††
Chlorides as NaCl mgs/100 cc serum	460.0	—	480.0***	—	—	631.0†††
Total proteins gms/100 cc serum	—	—	M—6.49† F—6.65†	—	7.44††	M—6.7 † F—6.98†
Albumin gms/100 cc serum	—	—	M—4.45† F—4.35†	—	—	M—4.2 † F—4.26†
Globulin gms/100 cc serum	—	—	M—2.12† F—2.35†	—	—	M—2.6 † F—2.75†
Albumin/Globulin ratio	—	—	M—2.2:1† F—1.9:1†	—	—	M—1.6:1† F—1.5:1†
Sodium mEq/L	—	—	—	—	147.0	140.0 †*
Potassium mEq/L	—	—	—	—	4.7	3.5 †*
pH	Celis and Cruz 1939 7.4–7.45	—	—	—	—	7.36-7.59†**

Methods used:

* Folin-Wu
** Myers-Bailey
*** Whitehorn
† Greenberg
†† Leitz-Folin Wu
††† Leitz-Sendroy
†* Bechman Flame Photometer
†** pH meter
††* Copper sulfate sp. gravity.

authors and the results are consistently higher in the older age groups. "Age trends of blood cholesterol were linear."[7]

From the comparisons made, it is found that our values for sugar and pH are not affected by sex and age.

COMPARISON OF VALUES BY VARIOUS AUTHORS

Table 4 shows the results obtained by other authors who have worked on blood among Filipinos giving the methods used and the number of cases studied. Table 5 presents normal values determined in other countries by American and European

[7] Keys, Kimura, et al., Am. Jour. Clin. Nutr. 5 (1957) 279.

TABLE 5.—*Comparison of normal blood chemistry values obtained by foreign authors.*

Constituent	Kolmer (American)	Todd and Sanford (American)	Gradwohl (American)	Leitz (American)	Wotton-King (British)	This series (Filipino)
Nonprotein nitrogen mgs/100 cc blood	25–40	25–30	25–35	25–35	29–43	26.3–38.3
Urea nitrogen mgs/100 cc blood	8–18	9–17	10–15	12–15	16–35	9.9–14.6
Uric acid mgs/100 cc blood	2–4	2–4	2–3.5	2–4	1.6–3.9	2.6–4
Creatinine mgs/100 cc blood	1–2	1–2	1–2	0.8–2	0.1–1.2	1.1–1.7
Total proteins gms/100 cc serum	6.4–8	6–8	6.5–8.2	6–8	6.7–7.7	5.1–8
Albumin gms/100 cc serum	4–5.5	3.5–5.6	3.3–5.5	4–6	4.4–5.3	3.5–4.9
Globulin gms/100 cc serum	1.5–3.4	1.3–3.2	1.5–3.5	1.2–2.8	1.9–2.8	2.1–3.3
Albumin/Globulin ratio	1.5–2.5:1	1.5–2.5:1	1.5–2.5:1	2.1–3.1	1.9–2.3:1	1.2–2.1:1
Sugar mgs/100 cc blood	80–110	90–120	80–120	80–120	68–96	83–107
Cholesterol mgs/100 cc blood	230–250	140–250	150–230	150–230	153–260	152–219
Total lipids mgs/100 cc	400–600	—	450–550	—	—	399–551
Sodium mEq/L	137–143	137–147	130–143	—	137–148	127–150
Potassium mEq/L	4.1–5.6	4.1–5.6	4.1–5.6	—	3.5–3.9	2.7–4.3
Chlorides as NaCl mgs/100 cc serum	570–620	550–650	570–620	570–620	454–526	569–691
Calcium mgs/100 cc serum	9–11	8.5–11.5	9–11	9.0–11.5	9.6–10.8	8.1–11.9
Total Bilirubin mgs/100 cc serum	0.1–0.25	0.5–1.5	0.1–0.8	0.2–0.8	0.1–0.5	0.13–0.36
Icterus index units	4–6	2.5–5	4–6	4–6	—	3.6–6.2
Amylase units	—	—	80–150	80–180	91–163	78–135
Lipase cc of N/20 NaOH	1–1.5	0.2–1.5	0.5–1.5	—	—	0.2–0.9
Thymol turbidity units	—	—	0.4–4.0	0.4–4	—	0.9–3.8
Alkaline phosphatase units	1.5–4	1–5	1.5–4.0	1.5–4	4.5–9.5	1.3–4.7
Acid phosphatase units	1.5–4	2–3	1.5–4.0	1–4	1.2–3.1	0.6–3.2
Phosphates mgs/100 cc serum	2–5	3–4	3.7–5.0	3–4.5	—	2.2–4
CO_2 combining power vols. per cent	55–65	40–55	55.0–75.0	50–75	25–31	48–74
pH	7.3–7.5	7.3–7.5	7.4–7.9	—	—	7.36–7.59
S G O T units	—	Americans	8.0–40.0	—	—	9–33.5
S G P T units	—	do	5.0–35.0	—	—	8–26

authors for a comparison with our values. It will be noticed that most of our values are about the same as, and some slightly lower than, those obtained abroad. Our chlorides and calcium, however, are found to be slightly higher.

SUMMARY

1. Blood of 1,314 normal Filipinos, aged 15 to 68, were analyzed for the various chemical constituents.

2. The range of normal values with percentage is given.

TABLE 6.—*Normal values for blood chemistry among Filipinos.*

Constituent	Mean	Standard deviation	10 to 90 percentile
Nonprotein nitrogen mgs/100 cc blood	32.1	± 4.4	26.3–38.3
Urea nitrogen mgs/100 cc blood	12.6	± 1.9	9.9–14.6
Uric acid mgs/100 cc blood	3.25	± 0.6	2.6–4.0
Creatinine mgs/100 cc blood	1.38	± 0.25	1.1–1.7
Total proteins gms/100 cc serum	6.9	± 0.8	5.1–8.0
Albumin gms/100 cc serum	4.2	± 0.5	3.5–4.9
Globulin gms/100 cc serum	2.7	± 0.5	2.1–3.3
Albumin/globulin ratio	1.6:1	± 0.4	1.2:1–2.1:1
Sugar mgs/100 cc blood	95.8	± 9.0	83.0–107.0
Cholesterol mgs/100 cc blood	184.0	±26.0	152.0–219.0
Sodium mEq/L	140.0	± 8.4	127.0–150.0
Potassium mEq/L	3.45	± 0.6	2.7–4.3
Chlorides as NaCl mgs/100 cc	631.0	±49.0	569.0–691.0
Calcium mgs/100 cc	9.7	± 1.3	8.1–12.0
Total bilirubin mgs/100 cc	0.27	± 0.1	0.13–0.36
Icterus index units	5.1	± 1.1	3.6–6.2
Amylase units	96.0	±15.0	78.0–135.0
Lipase cc of N/20 NaOH	0.55	± 0.3	0.2–0.9
Thymol turbidity units	2.79	± 1.1	0.9–3.8
Alkaline phosphatase units	2.98	± 1.2	1.3–4.7
Acid phosphatase units	1.65	± 1.0	0.6–3.2
Phosphates mgs/100 cc	2.96	± 0.5	2.2–4.0
CO combining power vols. per cent	60.0	±10.0	48.0–74.0
S G O T units	20.0	± 9.0	9.0–34.0
S G P T units	17.0	± 7.0	8.0–26.0
Total lipids mgs/100 cc	473.0	±59.0	399.0–551.0
pH	7.47	± 0.1	7.36–7.59

3. A comparison of values for males and females has been made.

4. Values in the different age groups have been reported and the differences thereby noted.

5. The values reported by other authors are also presented.

ACKNOWLEDGMENTS

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AMINO-ACID COMPOSITION OF SOME PHILIPPINE PLANT FOODS*

By Isabel Concepcion[1] and Iluminada S. Cruz[2]

SEVEN TEXT FIGURES

It is now generally recognized and accepted that the requirement for protein in human and in animal nutrition is essentially a requirement for the essential amino acids that can be supplied by food. Present-day emphasis on protein quality rather than total quantity has stimulated the accumulation of data on the amino-acid composition of foods. These data may serve as a basis for practical nutrition programs in regions where the amino-acid contribution of staple foods is an important dietary factor due to limited income or faulty dietary habits.

The results of the three regional nutrition surveys conducted by the Food and Nutrition Research Center (formerly Institute of Nutrition) in 1957 and 1958(12, 41) tend to show that protein deficiency is not a major problem in the surveyed areas. However, a great proportion of the good sources of protein in this country have to be imported. Meat, poultry, fish, eggs, and milk are primarily animal protein foods and as such represent the most concentrated sources of amino acids that we have in the average diet. They are very expensive and are not within the reach of low-income families, who have to depend on cheaper sources of protein.

Many of our local beans and seeds have high protein values(22) but we have little information on their amino-acid content. There is very little data available(8, 16, 17) on the dietary essential amino-acid content of local plants for use in the determination of their supplementary value to the quality of the protein in the average Filipino diet. This study was therefore undertaken to investigate the essential amino-acid composition of some local plant foods which have high protein values.

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[1] Assistant Scientist and Assistant Laboratory Supervisor, Food and Nutrition Research Center, National Institute of Science and Technology, Manila.

[2] Laboratory Technician, National Science Development Board, Manila.

REVIEW OF LITERATURE

Comprehensive investigations on the amino-acid composition of foods as such, instead of on purified proteins from foods, have been made possible since 1943 with the development of microbiological assay procedures.(25, 26, 27, 35, 36, 44) The technique and apparatus for a micromethod which uses a total volume of 0.2 ml have been described by Henderson, et al.(30) A uniform medium was developed by Henderson and Snell(29) for the determination of ten essential amino acids with various organisms.

The essential amino-acid content of vegetable foodstuffs have been investigated by several workers.(1, 4, 5, 14, 31, 42) Belton and Hoover(7) determined eighteen amino acids in the mung bean hydrolysate by chemical and microbiological methods. Block and Bolling(9) have compiled the amino-acid composition of protein and foods reported by different research workers. Schweigert(43) summarized the values for ten essential amino acids obtained by several investigators for the analysis of some animal and plant foods. Balasubramian, et al.(3) reported the lysine, methionine, phenylalanine and histidine content of some Indian cereals. Edwards and co-workers(20) investigated the cystine, tyrosine and ten essential amino-acid contents of selected foods. Navia, et al.(38) reported the tryptophan, methionine and cystine contents of Cuban foods of vegetable origin. The tryptophan, methionine and cystine of 21 pulses and cereals collected in different parts of the Eastern Mediterranean, Southeast Asia and Western Pacific were analyzed by Jelliffe and coworkers.(34) Tables have been prepared by Orr and Watt(39) for estimating the amino-acid content of foods, dietaries and food supplies.

In the Philippines, Galvez and Santos(24) determined the arginine, histidine and lysine content of *kandule* (*Arius* spp.) using the Phinner and Rosedale modification of the Van Slyke method. Canapi(13) identified seven amino acids; namely, cystine, glycine, histidine, leucine, lysine, methionine and valine, in the acid hydrolysate of *dilis* by means of paper chromatography. Bendaña-Brown and co-workers(8) have determined by microbiological methods the essential amino acid and cystine content of some Philippine foods and feeds. The amino-acid content of two coconut protein fractions obtained from coconut milk was reported by Concepcion, et al.(15) in a preliminary study of their quality.

EXPERIMENTAL

Materials.—Foods of plant origin with protein content of 14 per cent or more and other foods which, although low in protein, are consumed in such great quantities that they contribute an appreciable amount of protein to the Filipino diet were chosen for analysis. These foods included 18 cereals, 7 tubers, 20 beans and seeds, and 5 nuts. Food yeast (*Torulopsis utilis*) was also included to investigate the possibility of using it as a protein supplement.

The cereals, beans and seeds were obtained from the Bureau of Plant Industry. The tubers and peanuts were purchased from various markets and grocery stores in Manila and Pasay City. The samples of coconut flour and copra cake came from the Philippine Coconut Administration and the food yeast was prepared by the former Biological Division of the National Institute of Science and Technology.

Preparation of samples.—Fresh tubers were peeled, cut into fine strips and dried in the sun or air until crisp. Then they were finely ground in a Wiley mill. Cereals, dried beans and seeds were also ground in the same mill. The powdered samples were sieved and placed in glass bottles with plastic covers and kept in the refrigerator. Samples of soybean and peanut, which contained plenty of fat, had been defatted with anhydrous ether in a Soxhlet extraction apparatus before their proximate composition was determined and the defatted samples were used for amino-acid analysis. The carbohydrate of cereals and tubers was not removed before hydrolysis.

Proximate analysis.—Moisture, ether extract and ash were determined on the powdered and defatted (for soybean and peanut only) samples by the methods of the Association of Official Agricultural Chemists.(2) Nitrogen was determined according to the method of Hamilton and Simpson.(28)

Hydrolysis for amino-acid analysis.—The food samples were hydrolyzed with hydrochloric acid in accordance with the method of Block, et al.(10) which was previously described for coconut protein.(15) This hydrolysate was used for the assay of eleven amino acids; namely, arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. Enzyme hydrolysis(6) was used for the determination of tryptophan.

Assay procedure.—The experimental conditions employed in the microbiological determination of 12 amino acids are given in Table 1. The assay procedure is the same as that described by Concepcion, et al.(15) for coconut protein. Food samples

TABLE 1.—*Experimental conditions employed in the microbiological determination of amino acids.*

Amino acid	Concentration of standard solution γ/ml	Microorganism* used	Basal medium	Inoculum medium
1. l—Arginine	50	*Streptococcus fæcalis* #9790	Barton-Wright(6)	Fox, et al.(23)
2. l—Histidine	12	*Leuconostoc mesenteroides* P60	Horn, et al.(32)	Barton-Wright(6)
3. l—Lysine	50	*Leuconostoc mesenteroides* P60	Horn, et al.(32)	Barton-Wright(6)
4. l—Tyrosine	15	*Leuconostoc mesenteroides* P60	Horn, et al.(32)	Barton-Wright(6)
5. l—Tryptophan	6	*Lactobacillus arabinosus* 17-5	Barton-Wright(6)	Barton-Wright(6)
6. dl—Phenylalanine	48	*Leuconostoc mesenteroides* P60	Horn, et al.(32)	Barton-Wright(6)
7. l—Cystine	10	*Leuconostoc mesenteroides* P60	Lyman, et al.(30)	Barton-Wright(6)
8. dl—Methionine	24	*Leuconostoc mesenteroides* P60	Lyman, et al.(30)	Barton-Wright(6)
9. dl—Threonine	60	*Streptococcus fæcalis* #9790	Barton-Wright(6)	Fox, et al.(23)
10. dl—Leucine	60	*Lactobacillus arabinosus* 17-5	Barton-Wright(6)	Barton-Wright(6)
11. dl—Isoleucine	40	*Lactobacillus arabinosus* 17-5	Barton-Wright(6)	Barton-Wright(6)
12. dl-Valine	48	*Lactobacillus arabinosus* 17-5	Dunn, et al. (19)	Barton-Wright(6)

* Microorganisms were obtained from the American Type Culture Collection, Georgetown University School of Medicine, Washington, D.C., U.S.A.

were analyzed in duplicate using freshly prepared hydrolysates. Two or more hydrolysates of the same sample purchased at different times or places were prepared for analysis.

RESULTS AND DISCUSSION

Table 2 gives the average percentages of moisture, protein, fat, and ash for 51 foods obtained from the analysis of the dry samples. Protein values were above 10 per cent except for cereals, tubers, and coconut meat. Food yeast has the highest protein content, 43.83 per cent. Values for fat were below 5

TABLE 2.—*Moisture, protein, fat and ash contents of some Philippine plant foods.*

Plant food	Scientific name	Moisture gm	Protein gm	Fat gm	Ash gm
CEREALS		*Per cent*	*Per cent*	*Per cent*	*Per cent*
1. Corn, white, Bicol	*Zea mays* Linn.	11.09	9.81	2.64	1.38
2. Corn, meal, white	*Zea mays* Linn.	12.74	6.82	1.81	1.59
3. Corn, yellow, Calamba	*Zea mays* Linn.	10.54	8.17	1.35	1.66
4. Corn, yellow, Cuban	*Zea mays* Linn.	9.28	8.92	1.62	2.04
5. Corn, yellow (183 x A113)	*Zea mays* Linn.	10.20	10.71	—	1.69
6. Corn, yellow (A112 x A113)	*Zea mays* Linn.	11.98	9.81	0.88	1.84
7. Rice, Milfor	*Oryza sativa* Linn.	10.94	6.78	0.44	0.49
8. Rice, Tjeremas	*Oryza sativa* Linn.	11.32	5.47	0.42	0.98
9. Rice, Fortuna	*Oryza sativa* Linn.	10.85	7.08	0.45	0.77
10. Rice, Peta	*Oryza sativa* Linn.	11.56	7.97	0.55	1.10
11. Rice, Elon-elon	*Oryza sativa* Linn.	10.33	7.26	0.51	1.14
12. Rice, Palawan	*Oryza sativa* Linn.	11.17	8.69	0.40	1.44
13. Rice, Milagrosa	*Oryza sativa* Linn.	10.16	7.02	0.45	0.54
14. Rice, Tjahaja	*Oryza sativa* Linn.	11.05	6.66	1.24	1.09
15. Rice, Raminad str. 3	*Oryza sativa* Linn.	10.04	7.26	0.48	0.93
16. Rice, Wagwag	*Oryza sativa* Linn.	10.06	6.72	0.44	1.23
17. Rice, Seraup Besar	*Oryza sativa* Linn.	9.15	6.55	1.04	0.75
18. Rice, S. Kechil	*Oryza sativa* Linn.	10.79	6.72	0.76	1.01
TUBERS					
1. Cassava, fresh	*Manihot utilissima* (Pohl.)	10.25	1.01	0.71	1.38
2. Cassava, grits	*Manihot utilissima* (Pohl.)	9.31	1.58	0.39	0.67
3. Sweet potato, purple	*Ipomoea batatas* (Linn.)	8.99	3.08	1.06	2.28
4. Sweet potato, white (3 samples)	*Ipomoea batatas* (Linn.)	10.29	2.00	0.57	2.62
5. Sweet potato, yellow	*Ipomoea batatas* (Linn.)	7.52	3.01	1.50	2.29
6. Taro (gabi)	*Colocasia esculentum* (Linn.) Schott and End.	9.55	4.77	1.00	3.34
7. Yam (ubi)	*Dioscorea alata* Linn.	10.17	6.25	1.23	2.91
BEANS AND SEEDS					
1. Bountiful bean (2 samples)	*Phaseolus vulgaris* Linn.	13.51	17.41	1.70	3.68
2. Lima bean, white (2 samples)	*Phaseolus lunatus* Linn.	11.30	14.36	1.82	3.88
3. Navy bean	*Phaseolus vulgaris* Linn.	13.07	15.64	1.99	3.58
4. Red Kidney bean (2 samples)	*Phaseolus vulgaris* Linn.	12.06	15.50	1.80	3.56

Table 2.—*Moisture, protein, fat and ash contents of some Philippine plant foods*—Continued.

Plant food	Scientific name	Moisture gm	Protein gm	Fat gm	Ash gm
		Per cent	*Per cent*	*Per cent*	*Per cent*
5. Chickpea (2 samples)	*Cicer arietinum* Linn.	11.42	14.55	1.79	2.55
6. Cowpea, black paayap (2 samples)	*Vigna sinensis* (Linn.) Savi	12.17	17.92	1.00	2.98
7. Cowpea, black kibal	*Vigna sinensis* (Linn.) Savi	11.79	12.19	1.46	3.41
8. Cowpea, New Era	*Vigna sinensis* (Linn.) Savi	15.15	20.99	1.58	3.55
9. Cowpea, white paayap (2 samples)	*Vigna sinensis* (Linn.) Savi	13.16	16.33	1.92	2.97
10. Cowpea, white kibal	*Vigna sinensis* (Linn.) Savi	11.08	17.70	1.59	3.58
11. Mung bean, green (2 samples)	*Phaseolus aureus* Roxb.	10.00	19.77	1.23	3.48
12. Mung bean, red (2 samples)	*Phaseolus aureus* Roxb.	13.09	16.70	0.68	3.01
13. Mung bean, yellow (2 samples)	*Phaseolus aureus* Roxb.	11.61	23.11	1.12	3.44
14. Pigeon pea, red (kadyos) ..	*Cajanus cajan* (Linn.) Millsp.	10.33	12.67	1.77	3.12
15. Pigeon pea, white (kadyos)	*Cajanus cajan* (Linn.) Millsp.	12.80	14.05	1.46	3.74
16. Soybean (2 samples)	*Glycine max* (Linn.) Merr.	9.36	31.38	17.74	4.39
17. Soybean, defatted (2 samples)	*Glycine max* (Linn.) Merr.	8.97	31.75	1.67	4.99
18. Tapilan (2 samples)	*Phaseolus calcaratus* Roxb.	10.91	13.76	1.10	3.84
19. Yard-long bean, red (sitaw)	*Vigna sinensis* var. *sesquipedalis* Fruw.	13.38	21.62	1.96	3.18
20. Yard-long bean (sitaw Singapore)	*Vigna sinensis* var. *sesquipedalis* Fruw.	12.50	20.99	2.00	3.38
NUTS					
1. Peanut, raw, defatted (2 samples)	*Arachis hypogæa* (Linn.)	9.84	39.07	1.48	4.15
2. Peanut, roasted, defatted (2 samples)	*Arachis hypogæa* (Linn.)	7.56	35.54	1.69	4.36
3. Coconut meat (sapal)	*Cocos nucifera* (Linn.)	5.04	5.62	2.15	1.06
4. Coconut flour (2 samples)	*Cocos nucifera* (Linn.)	7.56	11.97	20.10	4.05
5. Copra cake	*Cocos nucifera* (Linn.)	6.61	13.79	4.85	4.00
MISCELLANEOUS					
1. Food yeast	*Torulopsis utilis*	8.98	43.83	3.58	8.37

per cent except for soybeans (not defatted) and coconut flour. It should be noted, however, that the peanuts and one soybean sample had been defatted before an analysis of the fat was made. The coconut meat used in this study was the pressed cake obtained after the extraction of coconut milk, hence it had low fat content. Moisture values ranged from 5.04 per cent for coconut meat to 18.38 per cent for red yard-long bean (*sitaw*). Ash content varied from 0.49 per cent to 8.37 per cent.

Data on the amount of eight essential and four nonessential amino acids in foods are presented in Table 3. In order to facilitate comparison between the amino-acid values obtained in this study and those of other investigators, the Block and Bolling(9) method was used in the mode of presentation as there is considerable variation in the crude protein content of different foods. Accordingly, all the values were calculated to gm of amino acid per 100 gm of crude protein or gm amino acid per 16 gm nitrogen (moisture-free basis). Figures given are the average of duplicate or triplicate determinations of two or more hydrolysates of the same samples unless otherwise specified. Values for whole egg are included to serve as basis for the evaluation of the amino-acid content of the foods.(18, 37)

The amino-acid contents of the different varieties of white and yellow corn reported in this study were more or less the same. No outstanding difference was found in the amino-acid values of the twelve varieties of rice that were analyzed.

White cowpea (*paayap*) has slightly higher values than the black variety for eight amino acids, the histidine and tyrosine values were lower in the white variety, while similar values were obtained for cystine and phenylalanine in both varieties. Values for ten amino acids were higher for the white *kibal* than those for the black variety. The amino-acid content of red mung bean was higher than that for the yellow and green varieties except for lysine and tryptophan. All amino acids, except lysine, were found to be higher for the defatted soybean than for the original sample. No tryptophan was found in the tubers, this may be due to the presence of a relatively higher amount of carbohydrate than protein. Tryptophan was not determined in corn because when the method for enzyme hydrolysis was adopted there was no more sample left of the varieties used for the analysis of the other amino acids. All the amino-acid values, except isoleucine and lysine, of food yeast were found to be lower than those for whole egg.

TABLE 3.—*Amino-acid content of some Philippine plant foods.*
(In terms of 16 gm N, moisture-free basis.)

Plant food	Arginine	Cystine	Histidine	Isoleucine†	Leucine†	Lysine†	Methionine†	Phenylalanine†	Threonine†	Tryptophan†	Tyrosine	Valine†
CEREALS												
1. Corn white, Bicol	4.75	0.61	2.70	6.21	11.95	5.00	2.28	5.09	5.91	—	1.71	6.18
2. Corn meal, white	5.24	1.04	3.16	6.71	9.85	6.11	3.35	7.56	4.78	—	2.69	6.80
3. Corn, yellow, Calamba	5.29	0.85	3.73	5.13	12.49	6.22	2.41	5.67	7.69	—	1.06	5.82
4. Corn, yellow, Cuban	6.87	0.72	4.71	5.21	11.68	5.87	2.25	5.29	8.02	—	1.41	6.19
5. Corn, yellow (183 x A 113)	5.23	0.61	3.76	5.30	12.89	4.37	2.03	5.21	6.83	—	1.19	5.80
6. Corn, yellow (A 112 x A 113)	4.99	0.79	4.20	4.88	12.89	5.16	2.17	5.45	8.74	—	1.12	5.12
7. Rice—Milflor	10.58	1.03	4.86	6.72	9.40	5.71	4.61	3.54	4.75	0.19	1.93	7.69
8. Rice—Tjeremas	9.56	0.86	3.35	7.73	8.03	4.46	4.86	4.48	4.53	0.23	1.38	6.64
9. Rice—Fortuna	8.16	0.85	3.20	6.01	8.03	4.09	4.13	4.10	5.25	0.34	1.26	7.20
10. Rice—Peta	9.80	0.80	3.70	6.80	7.67	4.85	4.77	5.15	3.88	0.28	1.42	6.71
11. Rice—Elon elon	7.51	1.05	3.10	6.28	7.60	4.37	4.10	4.53	3.22	0.17	1.39	6.06
12. Rice—Palawan	10.29	0.86	3.84	6.13	7.77	4.10	4.41	5.61	3.88	0.55	1.66	6.23
13. Rice—Milagrosa	8.40	0.89	3.22	4.95	7.01	5.14	4.00	4.63	3.42	0.32	1.52	5.93
14. Rice—Tjahaja	10.27	0.61	3.01	6.07	7.21	4.60	4.24	3.70	3.55	0.29	1.49	6.03
15. Rice—Raminad	9.26	0.70	3.83	5.14	8.02	4.92	3.71	4.43	3.94	0.26	1.32	6.61
16. Rice—Wagwag	10.54	0.72	3.72	6.30	8.11	6.45	4.85	3.28	3.68	0.47	1.87	6.52
17. Rice—Seraup Besar	8.11	0.72	3.11	4.92	8.00	5.08	3.53	3.51	3.80	0.15	1.10	6.34
18. Rice—S. Kechil	10.37	0.87	3.79	6.40	8.35	5.34	4.24	3.04	4.22	0.50	1.62	7.27
TUBERS												
1. Cassava, fresh	5.18	0.10	2.17	3.95	5.41	8.70	1.69	3.23	3.60	0.0	0.96	4.19
2. Cassava, grits	5.75	0.16	1.72	3.16	4.53	6.82	1.72	2.70	3.24	0.0	0.86	3.59
3. Sweet potato, purple	5.46	0.14	2.65	5.56	6.45	8.77	3.28	5.32	4.83	0.0	0.99	6.75
4. Sweet potato, white (3 samples)	5.36	0.09	2.21	5.38	6.77	7.96	2.87	4.56	4.21	0.0	0.86	6.07
5. Sweet potato, yellow	5.12	0.07	2.36	5.19	6.23	7.22	3.01	5.59	4.12	0.0	0.62	6.19
6. Taro (gabi)	7.47	0.38	2.56	4.83	8.17	8.34	2.57	8.25	4.43	0.0	1.88	6.02
7. Yam (ubi)	7.30	0.16	2.18	4.48	6.06	6.01	2.72	5.15	3.38	0.0	1.08	4.79
BEANS AND SEEDS												
1. Bountiful bean (2 samples)	9.25	0.38	4.27	7.26	8.71	11.03	2.61	6.26	4.52	0.29	2.60	6.13
2. Lima bean, white (2 samples)	8.14	0.06	4.27	6.36	7.70	11.09	2.33	5.85	4.58	0.43	2.31	5.77

† Essential amino acid.

TABLE 3.—*Amino-acid content of some Philippine plant foods*—Continued.

Plant food	Arginine	Cystine	Histidine	Isoleucine†	Leucine†	Lysine†	Methionine†	Phenylalanine†	Threonine†	Tryptophan†	Tyrosine	Valine†
3. Navy bean	9.37	0.50	5.52	6.59	8.54	7.98	2.18	5.29	5.96	0.36	2.61	6.24
4. Red kidney bean (2 samples)	8.56	0.42	4.70	6.47	8.89	14.05	2.38	7.13	4.66	0.18	2.65	6.53
5. Chickpea (2 samples)	12.79	0.59	3.94	6.14	8.75	11.53	2.83	6.87	4.17	0.64	2.01	5.75
6. Cowpea, black, paayap (2 samples)	11.74	0.40	4.59	6.65	8.97	6.68	3.02	7.07	4.71	0.32	3.08	6.85
7. Cowpea, black kibal	10.22	0.43	4.66	6.80	9.11	7.03	3.19	3.94	5.23	0.25	3.11	7.20
8. Cowpea, New Era	9.27	0.21	3.67	6.98	8.73	6.02	1.97	3.80	5.53	0.17	1.87	6.03
9. Cowpea, white paayap (2 samples)	13.29	0.40	3.94	7.39	9.78	6.92	3.76	7.07	4.90	0.54	2.75	7.41
10. Cowpea, white kibal	12.53	0.46	5.86	7.74	9.67	6.42	3.38	4.28	4.80	0.58	3.44	7.55
11. Mung bean, green (2 samples)	9.76	0.26	3.09	5.15	7.49	14.26	2.35	5.70	3.26	0.74	2.55	5.89
12. Mung bean, red (2 samples)	12.11	0.56	4.23	6.34	8.88	12.80	3.11	6.75	4.61	0.32	2.65	6.62
13. Mung bean, yellow (2 samples)	7.40	0.15	2.83	4.89	6.75	10.18	2.21	4.91	3.65	0.82	1.95	5.19
14. Pigeon pea, red (kadyos)	10.76	0.57	5.15	6.94	9.96	7.92	3.26	3.79	4.21	0.24	2.00	7.49
15. Pigeon pea, white (kadyos)	9.93	0.70	7.60	6.74	9.43	8.68	2.74	5.10	4.19	0.36	1.93	6.33
16. Soybean (2 samples)	9.13	0.53	3.41	6.20	6.07	10.92	2.20	4.77	4.03	0.70	2.65	5.72
17. Soybean, defatted (2 samples)	13.75	1.04	3.70	6.54	8.42	8.87	3.24	5.59	4.08	0.83	3.39	6.56
18. Tapilan (2 samples)	8.82	0.42	5.23	6.17	9.72	12.71	3.84	7.43	4.72	0.35	2.81	6.94
19. Yard-long bean, red (sitaw)	7.47	0.27	4.75	6.14	5.58	7.40	1.77	3.96	3.32	0.18	1.65	7.11
20. Yard-long bean, (sitaw Singapore)	11.01	0.39	5.38	7.35	8.84	6.71	3.05	5.69	5.54	0.38	2.68	6.03
NUTS												
1. Peanut, raw, defatted (2 samples)	18.05	0.81	3.63	5.04	7.36	5.52	2.21	6.07	3.39	1.88	3.66	5.62
2. Peanut, roasted, defatted (2 samples)	16.13	0.72	3.39	5.40	7.24	5.48	2.51	7.70	3.29	0.78	3.60	6.85
3. Coconut meat (sapal)	10.78	0.25	2.03	4.66	7.79	5.54	2.54	2.94	3.38	0.48	0.99	5.73
4. Coconut flour (2 samples)	7.50	0.14	0.80	4.28	6.69	2.21	1.32	2.90	2.18	0.20	0.68	4.20
5. Copra cake	10.78	0.70	1.69	3.40	5.61	5.14	2.58	3.45	2.86	0.0	1.74	5.33
MISCELLANEOUS												
1. Food yeast	3.97	0.47	1.86	5.76	5.73	9.34	2.24	4.94	4.54	0.64	0.59	5.31
2. Whole egg*	6.6	2.4	2.4	7.7	9.2	7.0	4.0	6.3	4.3	1.5	5.0	7.2

* Oser, Journ. Amer. Diet. Assoc. **27** (1951) 396-402.
† Essential amino acid.

The protein quality of a food can be estimated from its amino-acid composition by comparison with the amino acids of whole-egg protein. Mitchell and Block(37) used a chemical scoring system based on the single essential amino acid which is most deficient compared to whole-egg protein. Good correlation was found between the chemical scores and the biologic values in a series of 23 proteins. The protein score can also be obtained by comparison with the FAO provisional pattern of essential amino acids.(21) The figures of this pattern were derived from average minimal requirements for essential amino acids based on experimental data with tryptophan taken as unity. It has been shown that food and food mixtures with a biological value of 70 or more are satisfactory for growth and maintenance.

Oser(40) believes that protein quality rating should be based on the contribution a protein makes with respect to all the essential amino acids rather than just to the most deficient because each essential amino acid is specific in its own right and all are equally essential. The percentage ratios of amino acids in food proteins ($N \times 6.25$) relative to their content in whole-egg protein are called "egg ratios" and these values can be plotted as nutritional egg-ratio curves. The curves are plotted for food proteins with a maximum of 100 per cent, i.e., any essential amino acid present in higher proportion than in whole-egg protein is considered equal, not superior, to the standard. The empirical rating is known as the "Essential Amino Acid Index" (EAA Index). It is obtained by dividing the sum of the logarithms of the ten "egg ratios" by 10 and taking the antilogarithm of this mean. Cystine and methionine are considered one essential amino acid; arginine and histidine are included until conclusive evidence of their more widespread dispensability is adduced.

The protein score was not applied here because the values obtained for tryptophan were below 1 (except raw defatted peanut). Block(11) observed that errors in the determination of tryptophan requirement will exaggerate the errors for all other amino acids. Therefore, the EAA Index was used for evaluating the protein quality of the foods reported.

Table 4 gives the egg ratio and essential amino-acid index of the plant foods reported in this study. The nutritional egg-ratio curves of these foods are shown in Figs. 1 to 7. The tryptophan of corn was not determined; and because only 7 amino acids were included, the EAA Index was not computed. The EAA Indices

TABLE 4.—*Egg ratios of amino acids in some plant proteins.* ($N \times 6.25$).

Plant food	Arginine	Cystine + Methionine	Histidine	Isoleucine	Leucine	Lysine	Phenyl-alanine	Threo-nine	Trypto-phan	Valine	EAA Index
CEREALS & FOOD YEAST											
1. White corn (ave.)	75.7	56.9	163.8	183.9	118.5	79.4	100.4	124.3	—	90.1	—
2. Yellow corn (ave.)	84.8	46.2	170.8	66.6	135.8	77.2	85.8	181.9	—	76.1	—
3. Rice (ave.)	142.4	80.0	148.3	80.0	86.2	80.6	66.2	93.3	20.7	91.7	80
4. Food yeast	60.2	42.3	75.5	74.8	62.3	133.4	78.4	105.6	42.7	73.8	71
TUBERS											
1. Cassava, fresh	78.5	28.0	90.4	51.3	58.8	124.3	51.3	83.7	0.0	58.2	42
2. Sweet potato (ave.)	80.5	49.3	100.0	69.8	70.5	113.6	81.9	102.0	0.0	88.0	53
3. Taro (gabi)	113.2	46.1	106.7	62.7	88.8	119.1	131.0	103.0	0.0	83.6	58
4. Yam (ubi)	110.6	45.0	90.8	58.2	65.9	85.9	81.7	78.6	0.0	66.5	48
BEANS, SEEDS AND NUTS											
1. Bountiful beans (ave.)	134.9	45.2	186.9	89.2	95.7	179.1	106.3	106.7	15.7	89.9	88
2. Navy beans	142.0	41.9	230.0	85.6	92.8	114.0	84.0	138.6	24.0	86.7	89
3. Lima beans	123.3	37.3	177.9	82.6	83.7	158.4	92.9	106.5	28.7	80.1	85
4. Cowpea, kibal (ave.)	172.3	58.3	219.2	94.4	102.1	96.1	65.2	116.6	27.7	102.4	92
5. Cowpea, paayap (ave.)	184.6	50.8	177.7	91.2	101.6	97.1	112.2	111.7	28.7	93.9	90
6. Yard-long bean, sitaw (ave.)	140.0	42.8	211.0	87.6	78.4	100.8	76.6	103.0	18.7	91.3	87
7. Pigeon pea, kadyos (ave.)	156.7	56.8	265.6	88.8	105.4	118.6	70.6	97.7	20.0	96.0	90
8. Chickpea	193.8	53.4	164.2	79.7	95.1	164.7	109.0	97.0	42.7	79.9	97
9. Mungbean (ave.)	147.8	45.0	141.0	70.9	83.8	177.3	91.9	89.3	41.8	81.9	88
10. Soybean (defatted)	208.3	66.9	154.2	84.9	91.5	126.7	88.7	94.9	55.3	91.1	99
11. Peanut (raw)	273.5	47.2	151.3	65.5	80.0	79.9	96.3	78.8	125.3	78.1	95
12. Tapilan	133.6	66.6	220.0	80.1	105.7	181.6	117.9	109.8	23.3	96.4	99
13. Copra cake	163.3	51.3	70.4	44.2	61.0	73.4	54.8	66.5	0.0	74.0	45
14. Coconut flour	113.6	22.8	33.3	55.6	72.7	31.6	46.0	50.7	13.3	58.3	43
15. Coconut meat	163.3	43.6	84.6	60.5	84.7	79.1	46.7	78.6	32.0	79.6	69

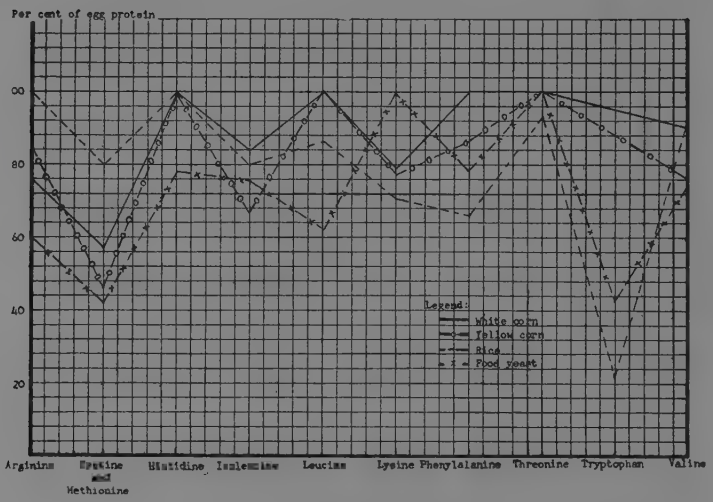

Fig. 1. Nutritional egg-ratio curves for rice, corn, and food yeast.

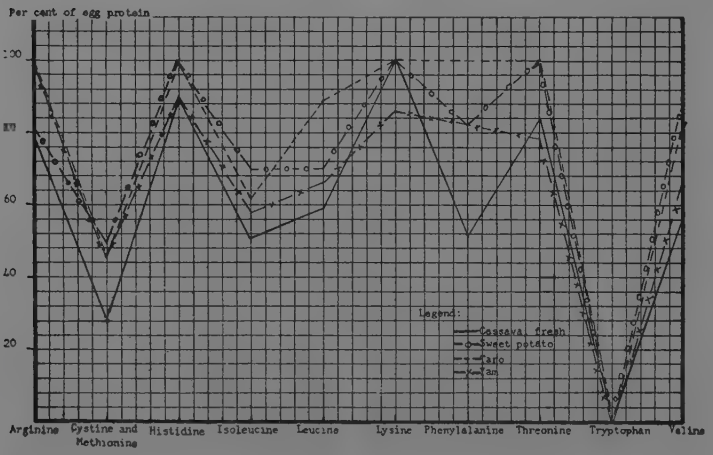

Fig. 2. Nutritional egg ratio curves for cassava, sweet potato, taro, and yam.

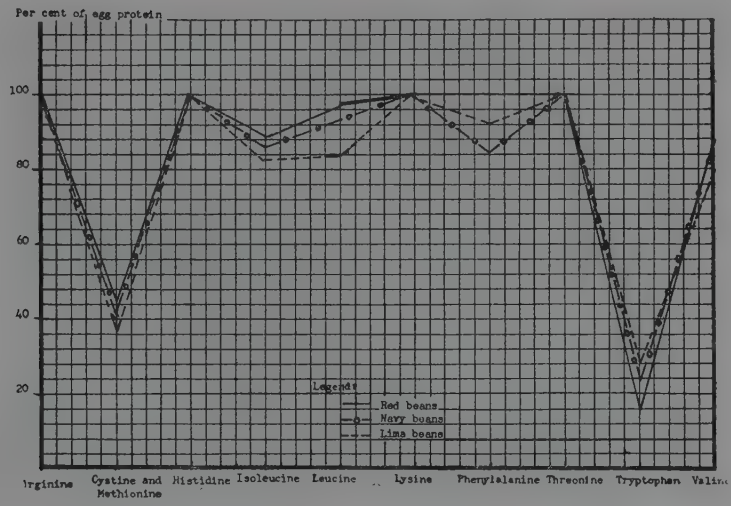

Fig. 3. Nutritional egg-ratio curves for red beans, navy beans, and lima beans.

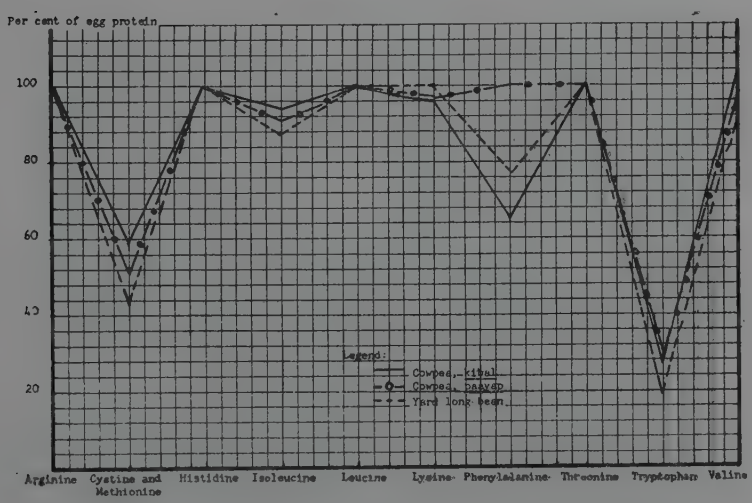

FIG. 4. Nutritional egg-ratio curves for cowpea (kibal and paayap) and yard-long bean.

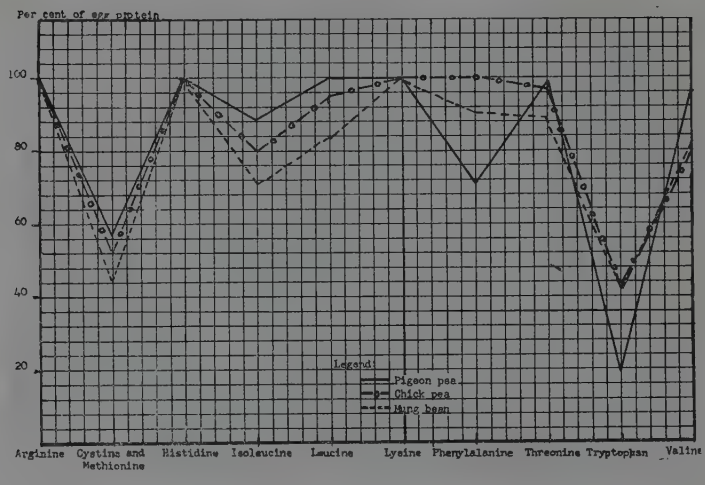

Fig. 5. Nutritional egg-ratio curves for pigeon pea, chick pea and mung bean.

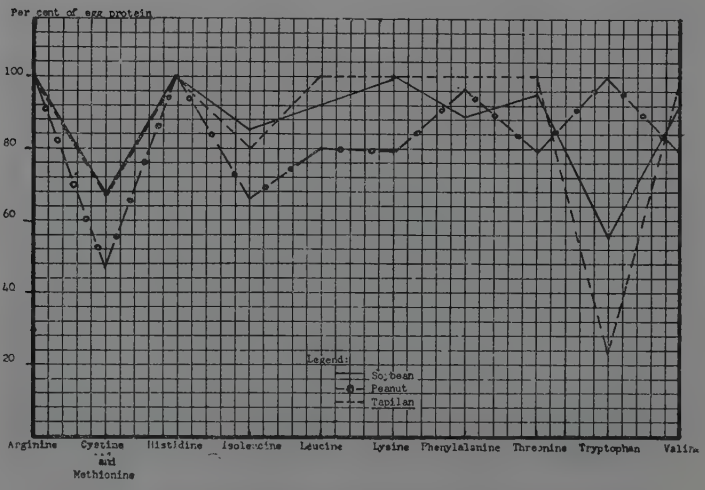

FIG. 6. Nutritional egg-ratio curves for soybean, peanut, and tapilan.

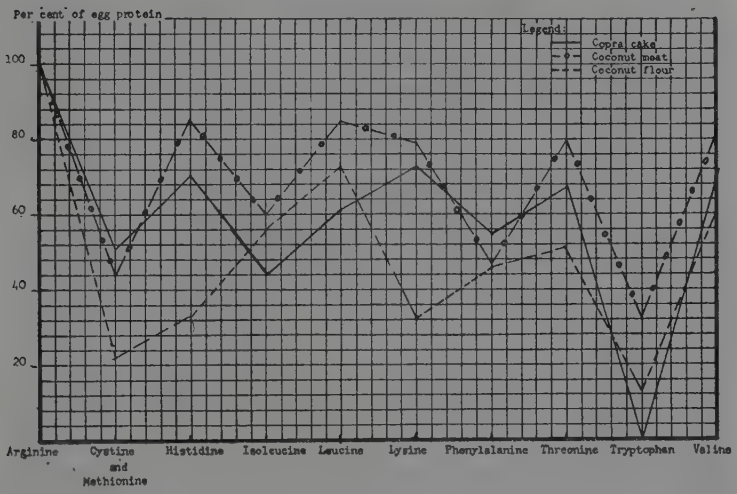

Fig. 7. Nutritional egg-ratio curves for copra cake, coconut meat and coconut flour.

ranged from 42 in fresh cassava to 99 in soybean (defatted) and in *tapilan*. In general, the values obtained were above 70; only the tubers, coconut flour, coconut meat, and copra cake had EAA Indices below 70. The nutritional egg-ratio curves usually had the same pattern for similar foods. Most of the foods had egg ratios of 100 per cent or more for arginine, histidine and threonine. Food yeast, fresh cassava, sweet potato, taro, beans and peas had lysine egg-ratios of more than 100 per cent.

The lowest egg-ratios were obtained for cystine + methionine and tryptophan in most of the plant foods analyzed in the present study. Other investigators (5, 30, 41) have found these to be the limiting amino acids in legumes, cereals and other vegetables.

Table 5 gives the amino-acid content of some plant foods reported in this study and by other investigators.(8, 20, 31, 33, 34, 38, 39, 42) In general, our values for most of the amino acids are comparable with those of other authors in spite of the difference in variety of samples and climatic conditions. However, lower values were obtained in this study for cystine, tryptophan and tyrosine in all foods (except peanuts). This may be due to the

TABLE 5.—*Comparison of amino-acid content of some plant foods as reported by the authors in this study with the findings of other investigators.* (*Gm amino-acid per 16 gm nitrogen, moisture-free basis.*)

Plant food	Arginine	Cystine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Tyrosine	Valine
CEREALS												
Corn, white (ave.)	4.75	0.61	2.70	6.21	11.95	5.00	2.28	5.09	5.91	—	1.71	6.18
Orr[39]	4.0	1.5	2.4	5.3	14.7	3.3	2.1	5.2	4.5	0.7	7.0	5.8
Navia[38]	—	—	—	—	—	2.7	2.3	—	—	0.6	—	—
Edwards[20]	5.0	1.8	2.3	4.1	11.9	2.2	2.3	5.0	4.7	0.3	4.0*	4.7
Rice (ave.)	9.40	0.83	3.56	6.16	7.93	4.93	4.29	4.17	4.01	0.31	1.50	6.60
Orr[39]	6.6	1.5	1.9	5.3	9.8	4.5	2.1	5.7	4.5	1.2	5.2	8.0
Jelliffe[34]	—	2.7	—	—	—	4.6	2.1	—	—	1.0	—	—
Horn, et al.**[33]	9.2	—	2.4	4.7	9.1	2.9	1.5	5.1	3.7	0.9	—	6.7
TUBERS												
Cassava, fresh	5.18	0.10	2.17	3.95	5.41	8.70	1.69	3.23	3.60	0.6	0.96	4.19
Ramachandran[42]	7.7	—	1.5	5.3	5.6	6.2	0.6	3.5	3.3	0.5	—	4.5
Sweet potato (ave.)	5.31	0.10	2.46	5.38	6.48	7.98	3.05	5.16	4.39	0.6	0.82	6.34
Hirsch[31]	5.2	—	2.4	5.1	5.6	5.5	2.6	6.1	5.7	2.1	—	9.3
Yam	7.30	0.16	2.18	4.48	6.06	6.01	2.72	5.15	3.38	0.6	1.08	4.79
Edwards[20]	3.4	1.6	1.6	4.0	5.7	4.1	1.1	5.4	3.4	1.3	4.5*	5.2
Ramachandran[42]	11.2	—	1.6	5.0	5.9	4.4	1.0	6.2	4.5	0.6		5.0
Taro	7.47	0.38	2.56	4.83	8.17	8.34	2.57	3.25	4.43	0.6	1.88	6.02
Navia[38]	—	—	—	—	—	19.7	4.6	—	—	3.3	—	—
BEANS AND SEEDS												
Lima bean	8.14	0.06	4.27	6.36	7.70	11.09	2.33	5.85	4.58	0.43	2.31	5.77
Orr[39]	7.3	1.7	3.7	6.6	9.5	7.6	1.8	6.8	5.4	1.1	3.0	7.2
Jelliffe[34]	—	2.1	—	—	—	7.5	1.9	—	—	0.8	—	—
Bendaña-Brown, et al.**[3]	2.15	0.69	5.70	5.19	8.20	4.70	0.98	5.10	4.27	1.48	—	5.26

* Alkaline hydrolysate
** Moisture- and ash-free.

TABLE 5.—*Comparison of amino-acid content of some plant foods*—Continued.

Plant food	Arginine	Cystine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylanine	Threonine	Tryptophan	Tyrosine	Valine
Navy beans	9.37	0.50	5.52	6.59	8.54	7.98	2.18	5.29	5.96	0.36	2.61	6.24
Orr[30]	6.8	1.1	3.2	6.4	9.7	8.4	1.1	6.2	4.9	1.1	4.4	6.9
Red kidney beans	8.56	0.42	4.70	6.47	8.89	14.05	2.38	7.13	4.66	0.18	2.65	6.53
Orr[30]	6.9	1.1	3.2	6.5	9.8	8.5	1.2	6.3	4.9	1.1	4.4	6.9
Jelliffe[34]	—	1.3	—	—	—	8.4	2.1	—	—	0.6	—	—
Bendaña-Brown, et al.**[8]	2.70	1.19	6.00	5.62	8.20	6.30	1.50	5.30	2.73	1.40	—	7.09
Chickpea	12.79	0.59	3.94	6.14	8.75	11.53	2.83	6.87	4.17	0.64	2.01	5.75
Orr[30]	8.3	1.6	3.0	6.4	8.3	7.7	1.5	5.4	4.0	0.9	3.7	5.5
Jelliffe[34]	—	2.3	—	—	—	9.1	2.0	—	—	0.6	—	—
Cowpea, paayap	12.52	0.40	4.27	7.02	9.38	6.60	3.39	7.07	4.81	0.43	2.92	7.13
Orr[30]	7.2	1.5	3.4	5.4	8.4	7.3	1.7	5.9	4.4	1.1	3.3	6.3
Bendaña-Brown, et al.**[8]	2.75	0.70	7.80	6.05	8.89	6.65	1.25	5.41	3.43	1.45	—	7.16
Mung bean, yellow	7.40	0.15	2.83	4.89	6.75	10.18	2.21	4.91	3.65	0.82	1.95	5.19
Bendaña-Brown, et al.**[8]	3.20	0.72	7.73	4.74	9.70	6.96	1.51	5.63	4.44	1.39	—	5.29
Jelliffe[34]	—	1.4	—	—	—	5.2	0.8	—	—	0.9	—	—
Soybean	9.13	0.53	3.41	6.20	6.07	10.92	2.20	4.77	4.03	0.70	2.65	5.72
Orr[30]	8.6	2.1	2.8	6.4	9.1	7.5	1.6	5.9	4.7	1.6	3.8	6.2
Jelliffe[34]	—	2.2	—	—	—	6.7	2.3	—	—	1.3	—	—
Tapilan	8.82	0.42	5.28	6.17	9.72	12.71	3.84	7.43	4.72	0.35	2.81	6.94
Bendaña-Brown, et al.**[8]	3.70	0.96	5.74	5.59	8.23	8.35	1.10	5.00	3.27	0.96	—	6.27
NUTS												
Roasted peanut	16.13	0.72	3.39	5.40	7.24	5.48	2.51	7.70	3.29	0.78	3.60	6.85
Orr[30]	12.6	1.8	2.9	4.8	7.1	4.2	1.0	6.0	3.2	1.3	4.2	4.6
Edwards[20]	10.7	1.0	2.1	3.5	6.5	2.8	1.0	5.4	2.8	0.9	4.6	5.8
Hirsch[31]	8.7	—	2.8	4.0	4.7	3.9	0.9	4.6	2.6	1.0	—	4.9
Peanuts, raw	18.05	0.81	3.63	5.04	7.36	5.52	2.21	6.07	3.39	1.88	3.66	5.62
Hirsch[31]	10.6	—	2.8	4.5	5.0	3.9	0.9	5.6	2.9	1.1	—	5.5
Navia[38]	—	—	—	—	—	4.7	1.1	—	—	1.3	—	—
Coconut meat	10.78	0.25	2.03	4.66	7.79	5.54	2.54	2.94	3.38	0.48	0.99	5.73
Navia[38]	—	—	—	—	—	9.4	3.41	—	—	—	—	—

** Moisture- and ash-free.

fact that carbohydrates were not removed from the foods before acid hydrolysis and because acid instead of alkali was used for the hydrolysis of samples in the analysis of tyrosine. Our results for arginine, lysine and methionine in beans and seeds are higher than those reported by Bendaña-Brown, et al.(8) except for the lysine of cowpea (paayap) where similar values have been obtained.

SUMMARY

Eight essential and four semiessential amino acids for man were determined microbiologically in 18 cereals, 7 tubers, 20 beans and seeds, 5 nuts and food yeast. Moisture, ether extract and ash were also analyzed in these foods before hydrolysis for amino-acid analysis.

The ranges of protein content are as follows: cereals 5.4 to 10.71 per cent; tubers, 1.01 to 6.25 per cent; beans and seeds, 12.19 to 31.75 per cent; nuts, 5.62 to 39.07 per cent; and food yeast, 43.83 per cent.

The egg ratio and essential amino-acid index (EAA index) of the plant foods reported in this study were used for evaluating their protein quality. With the exception of tubers, coconut meat, flour and copra cake, the EAA indices obtained were above 70. Most of the foods had egg ratios of over 100 per cent for arginine, histidine and threonine. The lowest egg ratios were obtained for cystine+methionine and tryptophan, which are reported in the literature to be the limiting amino acids in legumes, cereals, and other vegetables.

With the exception of cystine, tryptophan and tyrosine, the amino-acid values reported in this study are comparable with those of other authors in spite of differences in variety of samples and climatic conditions. Arginine, lysine, and methionine values for beans and seeds (except for lysine in cowpea) are higher in this study than those obtained by Bendaña-Brown, et al.

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STUDIES ON PHILIPPINE MEDICINAL PLANTS, I

PAPER CHROMATOGRAPHY AND SPECTROPHOTOMETRIC DETERMINATION

By Gloria D. Manalo
University of the Philippines, Quezon City
and
Glory C. Lleander
National Science Development Board,[1] Manila

TWO TEXT FIGURES

The exhaustive surveys(1, 2) of phytochemistry in the Philippines reveal the urgent need for continued effort on the study of the chemical structure of plant constituents from Philippine medicinal materials. Recent contributions include completion of the studies on *Pycnarrhena manillensis* Vidal,(3) the pharmacodynamics of pycnamine(4) and morphological studies of *Alstonia macrophylla* Wall.(5)

The role of physico-chemical methods in the determination of chemical structure of plant constituents like alkaloids(6) cannot be overemphasized. The abstracts of the papers(7-11) read at the Symposium on Natural Products in Australia include the application of such analytical tools as Spectroscopy (Ultraviolet, Infrared, Raman and Nuclear Magnetic Resonance) and Mass Spectrometry. The continued use of physico-chemical methods in the analytical studies of pharmaceuticals and related drugs is summarized annually in the Analytical Reviews.(12-16)

This paper summarizes the application of paper chromatography in the separation and comparison of the alkaloids from the roots and stem bark of *Alstonia macrophylla* Wall and the glycosides of *Strophanthus cumingii* A.DC. The results of this study will help in further studies on the chemical structure of these plant constituents. The other part presents the results of the assay of Philippine drugs whose constituents have definite structure aside from the additional studies on its pharmacological activity. The method of determination chosen is one which is suitable for pharmaceutical preparations of alkaloids, like injections or pills or tablets.

[1] Project 2.3 Pharmaceutical and Pharmacological Research. Dr. Alfredo C. Santos, Project Director.

The use of ion-exchange resins for the purification of the alkaloids has been studied. Berberine from *Berberis barandana* Vidal and *Archangelisia flava* (L.) Merr., is precipitated as reineckate and estimated at 420 mμ and phæantharine from *Phæanthus ebracteolatus* (Presl) Merrill and pycnamine from *Pycnarrhena manillensis* Vidal as reineckate at 5.25 mμ. The total alkaloids based on pycnamine are determined in the crude drug, the tincture and an alcoholic concentrate using ultraviolet absorption of pycnamine at 282 mμ.

EXPERIMENTAL

Paper chromatography.—The glycosides of strophantus samples were extracted and chromatographed according to the procedure of Schindler and Reichstein(17) using Whatman filter paper No. 1. The glycosides were detected with *m*-dinitrobenzene.

The alkaloids of *Alstonia* were extracted by two methods. Method I consisted of extraction with an ammoniacal mixture of ether-chloroform (3:1). This was then purified by shaking with 2 per cent sulfuric acid and after alkalinifying the acid extract with ammonia, extracted again with ether. The residue from this extract was used in the development of the chormatogram.

In Method II, the drug was exhausted with alcohol. The resinous matter was removed by treatment of the concentrated alcoholic extract with acidulated water and filtration of the precipitated resins. The acid filtrate was alkalinified and shaken with ether. For further purification, this ether extract was shaken with 2 per cent sulfuric acid and later the alkaloids taken up with ether. The chromatogram was developed following the method of Santos, G. A.(3) and using Dragendorf reagent for detection.

The different solvent systems and modifications of the ascending technique are shown in the figures.

Spectrophotometric determination.—Berberine was isolated by Santos, M.(18) and phæantharine and pycnamine by Santos, G. A.(3, 19) The reineckates of the alkaloids were precipitated from an acid solution of the bases with ammonium reineckate. The precipitate was filtered off using a sintered glass filter, washed with a solution of ammonium reineckate and dissolved in acetone. The absorbance of the acetone solution was measured in a Beckman spectrophotometer Model DU, the berberine re-

ineckate at 420 mμ and the other two salts at 525 mμ. A calibration curve was prepared for each of the three alkaloids.

The absorbance of pycnamine in ethanol was also measured at 282 mμ. Pycnarrhena, 10 gm of crude drug were exhausted with alcohol. After concentration of alcoholic extract to a convenient volume, an aliquot representing 1 gm of drug was digested in a water bath with Permutit Q (H^+ form) or Dowex 50 W x 4 (50-100 mesh). The alkaloids were eluted with ethanolic ammonia. Further purification was effected by passing the acidified eluate through a column of Permutit S_1 or Dowex 1 x 4 (50-100 mesh). The absorbance of each diluted eluate portion was measured at 282 mμ. Comparison was made with a calibration curve prepared from pycnamine. Blanks (drug marc) were treated similarly. Portions representing 1 gm of drug in the reserve percolate of the tincture (10 ml represent 1 gm drug) and the alcoholic concentrate (1 gm represents 7 gm drug) were analyzed by the same procedure.

RESULTS AND DISCUSSION

PAPER CHROMATOGRAPHY

Strophanthus glycosides.—Cardiotonic glycosides have been previously studied in this laboratory(20) and bark and leaf samples from different varieties of *Nerium oleander* Linn. were assayed by Baljet reaction.(21) Cosme and co-workers reported on the distribution of glycosides in *Thevetia peruviana* (Pers.) Merr. and *Nerium indicum* Mill.(22) A new cardiac glycoside, peruvoside from *Thevetia nerifolia* has been isolated by Kohli and Vohra.(23)

The chemical and pharmacodynamic investigation of *Strophanthus letei* Merr.(24) and the pharmacognosy of *Strophanthus cumingii* A.DC.(25) have been reported. A survey(26) of the innumerable species of *Strophanthus* revealed that neither of these two Philippine species has ever been investigated chemically. For the comparison of these materials with the reported species, paper chromatography is a most suitable method of analysis. Various conditions for a significant interpretation of chromatograms were determined.

Figs. A and B show the effect of time; Figs. B and C, the effect of solvent. The relation of the glycosides of *Strophanthus cumingii* to strophanthin and ouabain is shown in Fig. D and its separation into components in Fig. E. The corresponding

STROPHANTHUS GLYCOSIDES

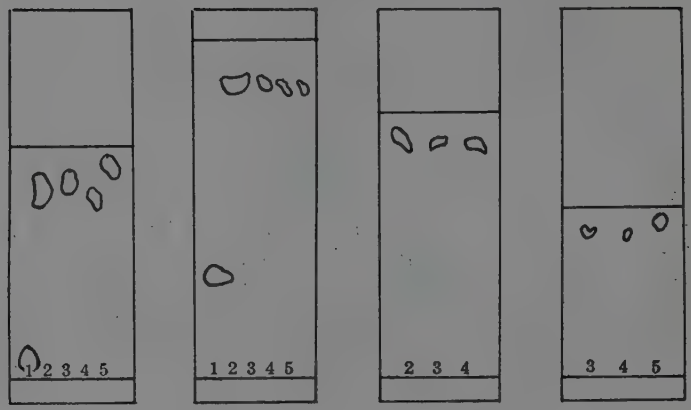

FIG. A FIG. B FIG. C FIG. D

FIG. A. Ethyl acetate-chloroform-water (10:2:5), 5 hours; B, ethyl acetate-chloroform-water (10:2:5), 24 hours; C, water saturated butanol-water saturated toluene (1:1), 23½ hours; D, ethyl acetate-chloroform-water (10:2:5), 8 hours.

1. Digitoxin, U.S.P. Reference Standard, 2. Commercial sample of strophanthus, 3. Strophantin tablets (Hypodermic) gr. 1/100, Burroughs Wellcome and Co., N.Y., 4. *Strophanthus cumingii* A.DC., 5. Ouobain, U.S.P. Reference Standard.

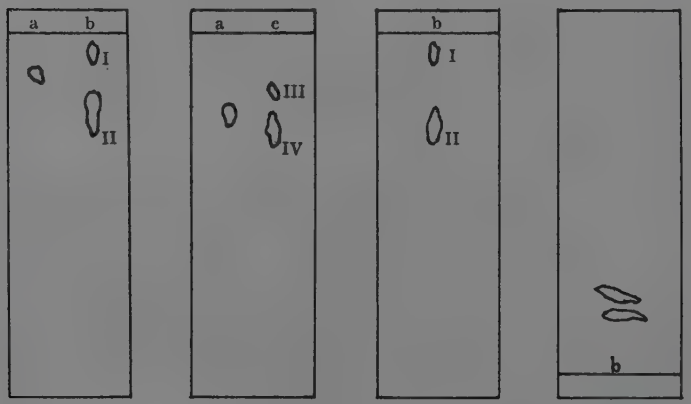

FIG. E FIG. F FIG. G FIG. H

FIG. E. Benzene-chloroform (7:5), 22½ hours; F, benzene-chloroform (5:7), 20 hours; G, benzene-chloroform (5:7), 25 hours; H, benzene-chloroform (7:5), 40 hours.

a. Strophantin tablet; b. *Strophanthus cumingii* A.DC.; c. commercial sample of strophanthus.

separation of the glycosides from a commercial sample of strophanthus is given in Fig. F. Strophanthin (a) was used as standard for comparison in this separation of the glycosides. Figs. G and H show the difference between the ascending and descending chromatography; and the effect of different proportions of the solvent system is shown in Figs. E and G.

A concise correlation of the results shown in the whole series of chromatograms of the cardiotonic glycosides emphasizes the need for finding out the best conditions necessary for the evaluation of the results. The choice of the solvent system is dependent on the purpose of the chromatography, i.e. whether any separation is desired or comparison is sufficient. The next consideration is the duration of the development of the chromatogram. Results indicated that for the samples studied the two solvent systems, Butanol-toluene and ethyl acetate-chloroform-water, did not effect any separation even by the two dimensional procedure. The only difference in the results with these two solvent systems was in the time for the development of chromatogram. Figs. A and D show that development for 3 to 5 hours was sufficient to indicate that the glycosides of the two strophanthus samples would be much more similar to strophantin than to ouabain and definitely different from digitoxin, using ethyl acetate-chloroform-water (10:2:5). Figs. E, F, G, and H indicate the efficiency of benzene-chloroform in effecting separation of the strophanthus glycosides. No appreciable difference in the separation is shown by changing the proportion from 5 : 7 to 7 : 5 for benzene-chloroform. On the other hand a big difference is shown between the ascending and descending techniques not only in the clearness of the separation but the duration of the development. While there is the two-dimensional procedure available for the separation, the uni-dimensional technique is most conveniently carried out.

A useful constant in the comparison and identification of substances by paper chromatography is the R_f value. Substances exhibiting (under the same conditions) different R_f values are different from one another; however, those having the same values are not necessarily the same. The four alkaloids of *Pycnarrhena manillensis*,(3) for example, were separated into nonphenolic and phenolic portions. Of these portions, pycnamine (phenolic) had the same R_f as phæanthin (nonphenolic) and berbamine (phenolic) about the same R_f as iso-

ALSTONIA ALKALOIDS

FIG. 1

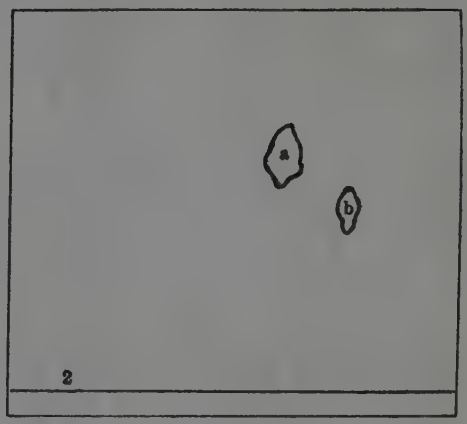

FIG. 2

FIG. 1. N/10 hydrochloric acid-10 per cent sodium sulfate (1:1), 6 hours. Extract from Method I of 1. stem bark, 2. roots.

2. N/100 hydrochloric acid-10 per cent sodium sulfate (1:1), 7 hours, first leg; 16 hours, second leg. Extract from Method I of roots.

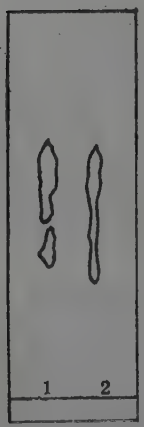

FIG. 3

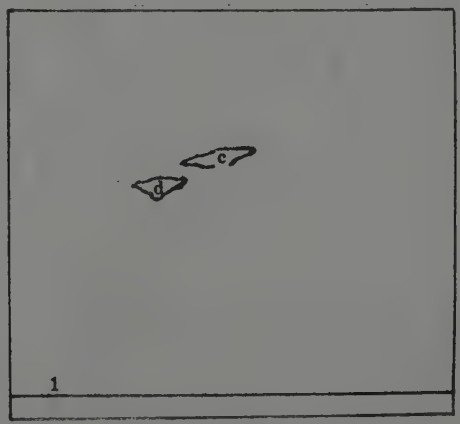

FIG. 4

FIG. 3. Butanol-acetic acid-water (4:1:5), 31 hours. Extract from Method II of 1. stem bark, 2. roots.

4. Butanol-acetic acid-water (4:1:5), 16 hours, first leg; 24 hours, second leg. Extract from Method I of stem bark.

tetrandine (nonphenolic). The nonphenolic bases are the corresponding methyl derivatives of the phenolic bases. In the chromatograms (Figs. E, F, G) showing the separation of the glycosides, the R_f value could not be determined since the papers were impregnated with formamide and thence the solvent front cannot be detected at all.

A wider separation was effected for the glycosides of *S. cumingii* (Figs. E and G, than for those of the commercial sample (Fig. F). The R_f values of the separated glycosides are not the same as that of strophanthin (Figs. E and F). This relation of the glycosides can be verified by chromatography of a mixture of each extract of the strophanthus samples with strophanthin. The blue color of the spots II and IV (Figs. E and F) was more intense than that of I and III. In a similar manner, for the same concentration of the extract from the commercial sample and *S. cumingii*, a darker blue spot was obtained from the former than from the latter.

Santos, J. K.(25) reported the close similarity of *Strophanthus cumingii* A.DC. to *Strophanthus wallichi* D. C. and other characteristics comparable to *S. divaricatus, S. caudatus* Kurz., and *S. erectus* Merr. The glycosides, caudoside and divaricoside, have been isolated from *S. divaricatus* (Lour.) Hook et Arn.(27) and *S. caudatus* (Burm. ex L.) Kurz.(28) Paper chromatography using chloroform as solvent for 8 hours showed that caudoside isolated from *S. caudatus* is the same as that from *S. wightiana* Wall and divaricoside, the same as that from *S. divaricatus*. However, using benzene-chloroform (5 : 7) divaricoside has a lower R_f than caudoside. Schindler and Reichstein(27) have reported that with benzene-chloroform (9 : 1) for 24 hours, caudoside and divaricoside show about the same R_f; while with chloroform for 8 hours, divaricoside has a little lower R_f than caudoside. Further, with these same glycosides, chloroform gives faster travel than benzene-chloroform. These two glycosides belong to the sarmentogenin group of strophanthus glycosides.(29)

Alstonia alkaloids.—Four alkaloids have been isolated by Sharp(30) from the stem bark of the Philippine species of *Alstonia macrophylla* Wall and an indole alkaloid, macrophylline, from the tertiary nonphenolic portion of the root alkaloids.(31) An exhaustive study of the pharmacology, with very meager data on the chemical nature, of an alkaloid named ma-

crophylline also has been reported by workers at the Araneta University.(36) In the present work, the alkaloids from the stem bark and roots, extracted by the two methods, were compared by paper chromatography.

The orange-red color of the spot (Fig. 1) from the stem bark is darker than that from the roots. Separation of the alkaloids of the roots with the aqueous acid-salt solvent is shown in the two-dimensional technique in Fig. 2. This same procedure did not effect any separation of the alkaloids from the stem bark. A less polar solvent system (butanol-acetic acid-water, 4:1:5) was then tried and the results are shown in Fig. 3. This revealed that while it took quite a long time to develop the chromatogram, uni-dimensional procedure effected a separation. To improve this the descending technique discussed for the glycosides can be resorted to. The separation of the alkaloids from the stem bark using this latter solvent system is shown in Fig. 4.

Examination of the chromatograms in Fig. 2 revealed that spot (a) is darker in color than spot (b); however, in Fig. 4 spot (c) is darker in color than spot (d). The results of these experiments will serve as a guide in the further studies of these alkaloids, which will be undertaken in this laboratory.

SPECTROPHOTOMETRIC DETERMINATION

The pharmacodynamics and completed studies on the chemistry of the isolated alkaloids from Philippine materials have made available these substances for clinical use. The pure alkaloids could be administered in the form of parenteral preparations and other pharmaceutical preparations containing small amounts of the active ingredient. This anticipated development in pharmaceutical manufacturing would necessitate assay meth-

TABLE 1.—*Spectrophotometric determination of the alkaloids.*

Sample	Alkaloid	Percentage*
Phaeanthus ebracteolatus (Presl) Merrill Crude drug	Phaeantharine	0.60
Pycnarrhena manillensis Vidal Crude drug	Total alkaloids (based on pycnamine)	2.4
Tincture (10 ml represents 1 gm drug)	do	0.28
Alcoholic concentrate (1 gm represents 7 gm drug)	do	14.0

* Average of duplicate results.

ods suitable for minute amounts. Of the various methods available, spectrophotometry was chosen. The usual procedure for the purification of alkaloids was improved by using ion-exchange resins. The extensive use of ion-exchangers for alkaloids is summarized by Calmon.(32) Dowex resins have been used by Brochmann-Hanasen(33) and Haberli and Beguin.(34)

Berberine hydrochloride has been assayed(35) using Amberlite IRC-50 and its absorption at 426 mμ. Although this alkaloid is highly colored yellow, conversion to its reineckate offers a much more sensitive determination. A solution containing as low as 0.05 mg/ml of berberine gave an optical density of 0.490. The reineckate of phæantharine and pycnamine gave maximum absorption at 525 mμ and the optical density was 0.237 for 0.6 mg/ml of phaeantharine and 0.184 for 0.6 mg/ml of pycnamine. The ultraviolet absorption at 282 mμ of pychamine offered a more sensitive estimation, 0.05 mg/ml of its solution gave an optical density of 0.560.

Permutit Q and Dowex 50/W x 4 (50 to 100 mesh) gave the same percentage of the total alkaloids (based on pycnamine) of Pycnarrhena. Dowex resin has the advantage in that the first portion of the eluate extracted most of the alkaloids present. For both resins four portions were collected. The assay of the alkaloidal drugs is shown in Table 1.

SUMMARY

The glycosides of *Strophanthus cumingii* A.DC. were compared with cardiotonic glycosides (digitoxin, ouabain and strophanthin) by paper chromatography. This physico-chemical analysis has shown the presence of at least two components of these glycosides using benzene chloroform as developing solvent. The alkaloids from stem bark and roots of *Alstonia macrophylla* Wall were compared with one another using two different solvent systems.

Berberine, phaeantharine (as reineckate) and pycnamine (absorption at 282 mμ) were determined by the spectrophotometric method. This is suitable for the assay of pharmaceutical preparations containing small amounts of these alkaloids. Further studies are necessary when such pharmaceutical preparations would be available.

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NOTES ON MOSQUITO BIONOMICS, I

COLONIZATION OF A. MINIMUS FLAVIROSTRIS AND A. VAGUS LIMOSUS IN AN OUTDOOR INSECTARY*

By Domingo Santiago
Department of Health, Manila

Several attempts made by various workers in the Philippines to colonize *A. minimus flavirostris* in indoor and outdoor insectaries did not produce encouraging results. The absence of reports on the cause or causes of failure of the experiments leaves the would-be reviewer in no position to know what procedures and technics to discard, what to try, what problems have been solved and what difficulties still stand between failure and success. He starts all over again the same procedure and technics, only to meet perhaps with the same disappointment as was experienced by his predecessors.

As suggested by successful foreign workers, there is a general procedure that can reasonably be followed; but such a procedure is subject to the application of a variety of technics which must be selected or modified to suit local conditions and different mosquito species.

This paper describes the procedure and technics followed in the colonization of *A. minimus flavirostris* and *A. vagus limosus*, the results obtained, and the observations gathered therefrom. The outdoor insectary method was followed and conducted in a seminatural condition which was provided with all conceivable ecological conveniences to simulate natural environment.

THE INSECTARY

A cubical cage 36 feet long, 12 feet wide, and 8 feet high was constructed over a portion of a small stream originating from a perennial spring nearby. The cage was covered all over with double-wall 18-mesh wire screen. One third of the cage, at one end, was roofed with galvanized iron sheets and protected on three enclosing sides, from the ground floor to the ceiling, with outer lumber walling. The protected space served to shelter

* This experiment was conducted at the Institute of Malariology, Division of Malaria, Bureau of Disease Control, Department of Health, Tala, Caloocan, Rizal Province.

a carabao placed inside the insectary every night to provide fresh blood meals to adult mosquitoes. It also served as a sheltering room for mosquitoes during windy and rainy days. In the remaining unshaded area, a small stream was made to run back and forth in parallel channels with sufficient lanes in-between for the insectary technician to step on. At six points along the stream margin and equidistant to each other, side still-water pockets were excavated to provide for larvæ safe zones away from direct stream current. Undercuts and small caves were also excavated along the stream banks to provide daytime resting places for adult mosquitoes. Extra earth-lined wooden boxes, which were kept wet, were posted in cage corners for the same purpose.

Spring water was allowed to flow continuously into the insectary stream through a 1½-inch diameter galvanized water pipe. The water pipe was provided with 70-mesh copper wire-screen at the inlet end to prevent the passage of larvæ breeding in spring water outside the insectary. The outflowing water was filtered again through a similarly screened water pipe placed at the outlet to block colony mosquito eggs and larvæ from escaping out. A portion of the roof adjoining the carabao shed, lengthwise and four feet wide, was raised to 14 feet and was roofed with panels of clear glass to provide swarming space for male mosquitoes. Creeping grasses and shrubs were planted along the sides within the insectary to create such natural environment that would make the mosquitoes feel so much at home.

PRE-COLONIZATION TESTS

To attract adult mosquitoes and induce them to force their way through a single-wall 18-mesh wire screen, a carabao bait was placed inside the insectary and left overnight with all doors securely locked. One *flavirostris*, one *limosus*, two *annularis*, and one *barbirostris manalangi* (all full-blooded females) were found inside the cage the next morning. In the following night, the same procedure was repeated. Two *limosus* and two *annularis* (all full-blooded females) were found again inside the cage the following morning. The screen walls and ceiling were then made of double-wall screen and tested again in the same way as before. For two consecutive nights the insectary was found negative for adult mosquitoes, which showed that double-wall 18-mesh wire screen was undoubtedly mosquito-proof.

Before the spring water was allowed to flow into the insectary stream the connecting water pipe was provided with a 70-mesh copper-wire screen filter. For one week the insectary stream was thoroughly searched but no first instar larva could be found. This showed that the screen was a larva-proof filter.

The spring water was tested to find out if it was suitable for anopheline breeding. This was done by removing the screen filter at the inlet end of the water pipe and the anopheline larvæ breeding in the spring water allowed to infiltrate and circulate freely in the insectary stream. In seven days 991 anopheline larvæ had infiltrated into the insectary stream.

A study of larval increase and decrease in the insectary stream revealed the following: For the first three days the high percentages of larval increase, from 32 to 66 per cent, were attributable to the first rush-in of accumulated larvæ in the spring for some time before the removal of the screen filter. Then the next three days the increase dropped from 29 to 5 per cent; this rate was considered normal.

The filtration of water was resumed to check further inroads of larvæ from spring into the insectary stream. Because of the fineness of the mesh of the screen filter, the volume of water passing through it was considerably reduced and consequently the water in the insectary stream dropped to two thirds of its original level. For the first three days the mortality among the larval population in the insectary stream was from 14 per cent to 26 per cent. For the next five days, after the flow of water had apparently settled down to normal; the mortality dropped from 7 to 1 per cent, which signified that the water was highly suitable for anopheline breeding.

Eight days after, the flow of water through the screen filter was partially blocked by the accumulation of corroded material, so much so, that the water level in the insectary stream started dropping rapidly. To raise it to normal height again the filter was cleared by rubbing away the clogging iron rust. This action gave rise to a sudden high concentration of iron compound and formation of a temporary thick, water-surface film which seemed to be detrimental to the breeding of anopheline larvæ. As a consequence, a high mortality rate was immediately observed among the young larvæ. The older ones appeared unaffected by such temporary chemical disturbance as indicated

by their continued development to the pupal stage. A few of the larvæ died after 22 days in the insectary stream.

The suitableness of spring water for breeding of *flavirostris* larvæ was further manifested by two facts: first, the larval density in the spring water outside the insectary was high; and secondly, the pupal percentage that had developed from larvæ in the insectary stream was comparable to that usually found in natural breeding places.

The pupal percentage in natural breeding places are from 2 to 15 per cent with an average of 8 per cent. (See Table 1.)

TABLE 1.—*Monthly total number of larvæ, pupæ, and pupal percentage collected from natural breeding places.*

Months		Larvæ	Pupæ	Pupæ
				Per cent
January	1956	141,979	13,428	9
February	1956	153,946	10,682	7
March	1956	110,193	9,543	8
April	1956	78,016	6,263	10
May	1956	78,177	4,149	5
June	1956	115,361	7,034	6
July	1956	70,563	5,442	7
August	1956	6,440	198	—
September	1956	2,341	——	7
October	1956	52,681	4,011	7
November	1956	87,161	6,359	2
December	1956	21,944	400	9
January	1957	82,638	7,357	14
February	1957	57,102	8,031	15
March	1957	57,972	9,148	3
April	1957	30,075	——	—
May	1957	42,242	——	—

A total of 107 or 11 per cent of 991 larvæ in the insectary stream had developed into pupæ. (See Table 2.)

COLONIZATION OF A. MINIMUS FLAVIROSTRIS

PROCEDURE

The larvæ and pupæ of *A. minimus flavirostris* were collected from natural breeding places. Only pupæ, together with those raised in the laboratory, were allowed to emerge into adults in-

side the insectary. Being a natural host, a carabao was placed inside the insectary every night to provide blood meals for adults. Insectary water analysis was conducted for one year from September, 1955 to August, 1956.

As a naturalistic method of mosquito colonization everything was left to nature including larval food which was provided by flowing water. To minimize unnecessary handling of colony larvæ and adults which might prove annoying or detrimental to normal development of mosquitoes no other elaborate technics had been applied, except for occasional or random counting of larvæ and pupæ and sorting of adults for the purpose of gathering vital statistical data.

RESULTS

(a) *Initial mosquito population.*—The initial *flavirostris* adult population fluctuated with the number of pupæ introduced in the insectary. The daily pupal averages, based on monthly totals from January, 1956 to March, 1957 were: minimum, 57; maximum, 434; average, 243. (See Table 3.)

(b) F_1 (*first filial*) *generation.*—The average daily number of adults by sexes and physiological condition of females, based on 33 days' observations during the height of adult production from March 23 to May 6, 1956, were as follows: (See Table 4.)

Average daily number of adults by sexes		Average daily number of assorted females	
	Per cent		Per cent
Males	27 or 44.26	Empty	34 or 57.62
Females	34 or 55.74	Engorged ...	15 or 25.43
		Gravid	10 or 16.95
Total	61 or 100.00	Total	59 or 100.00

The average daily number of larval (all stages) and pupal populations based on low adult production from July 13, 1956 to February 14, 1957 were as follows: (See Table 5.)

	Minimum	Maximum	Average
Average daily number of 1st instar larvæ	1	274	40
Average daily number of large larvæ	7	500	133
Average daily number of pupæ	1	16	4

TABLE 2.—*Pre-colonization test on larval and pupal development in the insectary stream.*

Date	No. of larvæ	No. of pupæ	Total	Increase	Increase	Decrease	Decrease
					Per cent		*Per cent*
December 2, 1955	117	—	117	56	32	—	—
December 3, 1955	233	—	233	139	60	—	—
December 4, 1955	372	—	372	245	66	—	—
December 5, 1955	617	—	617	31	5	—	—
December 6, 1955	648	—	648	190	29	—	—
December 7, 1955	838	—	838	153	18	—	—
December 8,* 1955	991	1	991	—	—	191	19
December 9, 1955	799	2	801	—	—	118	14
December 10, 1955	683	—	683	—	—	177	26
December 11, 1955	505	1	506	—	—	8	2
December 12, 1955	492	6	498	—	—	28	6
December 13, 1955	467	3	470	—	—	6	1
December 14, 1955	453	11	464	—	—	22	5
December 15, 1955	434	8	442	—	—	31	7
December 16, 1955	396	15	411	—	—	98	24
December 17, 1955	302	11	313	—	—	52	17
December 18, 1955	257	4	261	—	—	77	30
December 19, 1955	168	16	184	—	—	28	15
December 20, 1955	149	7	156	—	—	14	9
December 21, 1955	134	8	142	—	—	32	23
December 22, 1955	105	5	110	—	—	17	15
December 23, 1955	86	7	93	—	—	27	29
December 24, 1955	63	3	66	—	—	14	21
December 25, 1955	51	1	52	—	—	17	33
December 26, 1955	33	2	35	—	—	26	74
December 27, 1955	9	—	9	—	—	2	22
December 28, 1955	7	—	7	—	—	4	57
December 29, 1955	3	—	3	—	—	3	100

* Further entry of spring larvæ was blocked from this date.

DISCUSSION

Had the colonization started from larvæ it would have been impossible to differentiate the first filial from the introduced larvæ in the insectary stream. If it had started from adults caught wild it would have been difficult for us to collect a large number of imagoes during the long spell of dry season from harborages and even from carabao-baited traps, and the experiment would not have been carried out uninterruptedly. Under circumstances the most plausible procedure was to collect pupæ and introduce them in the insectary. It had the following advantages: First, the emerging adults easily got used to a restricted space in which they found themselves after emergence and became domesticated and much tamer than those in the open field; and secondly, as Buxton and Leeson(2) found out, the pairing of adults occurred soon after emergence and before the first meal. In nature it was apparently unusual to find a female with an empty spermatheca.

Technics for mating and results.—At the time when a large number of adults had accumulated in the insectary we spent several nights watching for possible swarming activities of males

TABLE 3.—*Number of pupæ introduced daily in the outdoor insectary from January 1956 to March 1957.*

Date	January 1956	February 1956	March 1956	April 1956	May 1956	June 1956	July 1956	August 1956	October 1956	November 1956	December 1956	January 1957	February 1957	March 1957
1	83	203	377	46	39	234	197	52	—	50	91	29	445	233
2	57	219	511	—	102	313	65	78	—	133	32	67	314	154
3	149	453	427	57	278	152	16	27	—	120	149	170	267	68
4	139	417	245	125	527	211	13	18	—	31	47	281	442	194
5	248	337	495	126	210	143	107	6	103	151	35	316	254	316
6	289	401	417	127	97	345	328	17	46	408	31	—	435	272
7	506	185	339	102	151	316	170	—	—	348	—	389	319	406
8	425	233	215	—	119	502	64	—	66	478	—	383	405	492
9	418	252	299	—	183	361	74	—	56	436	—	152	253	408
10	380	252	225	110	215	194	77	—	266	250	—	338	172	186
11	323	245	168	670	109	240	42	—	459	97	—	280	254	334
12	325	154	348	527	126	271	23	—	471	250	—	172	227	401
13	548	415	365	483	78	406	14	—	317	571	—	69	386	293
14	536	233	455	311	121	221	91	—	225	364	—	276	564	371
15	713	368	410	219	128	277	31	—	128	249	—	136	355	399
16	510	724	519	195	43	92	171	—	80	332	—	178	181	156
17	505	601	389	309	97	51	170	—	104	157	—	390	145	—
18	797	475	214	301	162	138	205	—	87	65	—	258	392	213
19	638	561	347	201	75	14	253	—	99	25	—	230	338	238
20	572	669	274	289	59	448	351	—	163	106	—	103	150	266
21	369	318	247	240	86	341	381	—	52	295	—	308	230	328
22	642	497	232	125	39	215	221	—	45	71	—	177	76	128
23	430	282	337	176	76	326	116	—	130	243	—	345	101	295
24	485	405	253	186	223	128	212	—	99	78	—	132	53	116
25	463	356	172	278	173	342	131	—	92	89	—	437	140	255
26	453	195	334	353	84	418	474	—	226	204	—	233	230	396
27	357	536	263	294	36	496	557	—	85	193	—	105	169	557
28	546	371	376	153	178	586	521	—	51	213	—	521	334	668
29	607	425	193	100	171	534	211	—	215	100	—	510	—	612
30	619	—	144	69	79	418	238	—	166	76	—	372	—	373
31	297	—	68	—	85	—	—	—	159	—	—	—	—	—
Total	13,428	10,682	9,543	6,253	4,149	7,034	5,442	198	4,011	6,359	400	7,357	8,031	9,148
Daily average	434	368	308	223	134	234	181	33	154	205	57	254	287	315

TABLE 4.—*Daily number of adults by sexes and by abdominal conditions of females in the outdoor insectary.*

Date	Male	Female	Empty female	Female engorged with blood	Gravid female	Total for both sexes
March 23, 1956	37	43	43	14	31	125
March 25, 1956	22	28	28	15	30	95
March 26, 1956	15	29	29	2	22	68
March 27, 1956	21	27	27	9	11	68
March 28, 1956	66	70	70	14	15	105
March 29, 1956	93	103	103	17	16	229
March 30, 1956	77	94	94	31	34	236
April 1, 1956	72	83	83	27	34	216
April 3, 1956	10	4	4	10	14	38
April 4, 1956	13	9	9	10	6	38
April 5, 1956	5	14	14	12	12	43
April 6, 1956	21	18	18	11	8	58
April 8, 1956	14	11	11	12	5	42
April 10, 1956	11	8	8	6	4	29
April 11, 1956	4	6	6	2	2	14
April 12, 1956	9	5	5	4	3	21
April 15, 1956	14	7	7	6	4	31
April 16, 1956	29	33	33	9	4	75
April 17, 1956	24	28	28	42	4	98
April 22, 1956	2	2	2	3	1	8
April 23, 1956	7	11	11	2	—	20
April 24, 1956	16	24	24	6	2	48
April 25, 1956	27	42	42	8	3	80
April 26, 1956	48	39	39	11	7	105
April 27, 1956	58	51	51	36	10	155
April 29, 1956	10	8	8	17	2	37
April 30, 1956	24	58	58	13	6	101
May 2, 1956	24	58	58	13	6	101
May 3, 1956	29	18	18	28	7	82
May 4, 1956	29	15	15	27	9	80
May 6, 1956	28	23	23	29	7	87
May 7, 1956	12	10	10	20	5	47
May 8, 1956	14	25	25	16	4	59
Total	885	1,004	1,004	482	328	2,699
Daily average ..	26.82	34.23	34.23	14.60	9.94	81.78

and eventual copulation or isolated pairing of males and females at evening twilight. Actual copulation has not been observed so far. Hanging objects of different colors at different heights or subdued blue light did not impress the shy males to start swarming activities. Perhaps the epigamic activities of *flavirostris* took place after evening twilight when it was too dark for ocular observations. The fact that 16.95 per cent of the females became gravid with viable eggs strongly indicated that copulation and fertilization of eggs had taken place inside the insectary.

Feeding of adults.—The average 25.43 per cent of engorged females in the insectary was an unmistakable sign that *flavirostris* had readily fed on carabao blood. This average was computed from the total number of adult population including recent emergences that were then not strong enough to fly and seek blood meals. It was a common observation in the insectary that *flavirostris* would start feeding on the carabao bait at about 8 p.m. At about 9:00 p.m. the majority were found already engorged with blood. No males nor females would feed on

TABLE 5.—*Number of colony larvæ and pupæ in the outdoor insectary stream from July 13, 1956 to February 14, 1957.*

Date	No. of 1st instar	No. of large larvæ	No. of pupæ	Total	Increased by	Increased	Decreased by	Decreased
						Per cent		*Per cent*
July 13, 1956	10	120	—	130	49	38	—	—
July 16, 1956	56	123	—	179	9	5	—	—
July 17, 1956	25	163	—	188	15	8	—	—
July 18, 1956	20	182	1	203	8	4	—	—
July 19, 1956	11	200	—	211	—	—	35	17
July 21, 1956	3	171	2	176	—	—	10	6
July 23, 1956	—	164	2	166	—	—	35	21
July 24, 1956	—	131	—	131	—	—	50	37
July 25, 1956	—	79	2	81	—	—	17	21
July 26, 1956	—	57	7	64	—	—	25	39
July 27, 1956	—	37	2	39	—	—	13	36
July 28, 1956	—	25	1	26	—	—	18	69
July 30, 1956	—	7	1	8	99	1225	—	—
July 31, 1956	—	107	—	107	96	89	—	—
August 1, 1956	75	128	—	203	—	—	101	49
August 2, 1956	22	80	—	102	9	9	—	—
August 3, 1956	15	96	—	111	—	—	2	2
August 4, 1956	19	90	—	109	—	—	5	5
August 6, 1956	26	78	—	104	11	11	—	—
August 7, 1956	13	102	—	115	60	52	—	—
August 8, 1956	37	138	—	175	—	—	54	31
August 9, 1956	—	121	—	121	7	6	—	—
August 10, 1956	1	126	1	128	—	—	27	21
August 11, 1956	—	101	—	101	—	—	13	13
August 13, 1956	—	84	4	88	—	—	18	20
August 14, 1956	12	56	2	70	—	—	13	19
August 15, 1956	7	50	—	57	26	46	—	—
August 16, 1956	16	65	2	83	20	24	—	—
August 17, 1956	24	76	3	103	—	—	3	3
August 18, 1956	5	94	1	100	—	—	10	10
August 20, 1956	—	88	2	90	75	83	—	—
August 22, 1956	33	114	15	162	—	—	77	48
August 23, 1956	8	66	11	85	—	—	10	12
August 25, 1956	14	61	—	75	—	—	34	45
August 27, 1956	—	40	1	41	7	17	—	—
August 31, 1956	8	37	3	48	—	—	—	—
September, 1956			No observations					
October 13, 1956	84	175	—	259	—	—	19	7
October 15, 1956	40	193	7	240	20	8	—	—
October 16, 1956	20	236	4	260	—	—	2	8
October 17, 1956	12	243	3	258	—	—	82	32
October 18, 1956	5	167		176	—	—	30	17
October 19, 1956	—	143		146	—	—	15	10
October 20, 1956	—	129	6	131	—	—	13	10
October 22, 1956	—	112	7	118	—	—	30	25
October 23, 1956	—	81	5	88	2	2	—	—
October 24, 1956	6	77	2	90	—	—	34	38
October 25, 1956	1	50	5	56	—	—	19	34
October 26, 1956	—	33	4	37	3	8	—	—
October 27, 1956	—	34	6	40	—	—	16	04
October 29, 1956	—	22	2	24	—	—	4	17
October 30, 1956	—	17	3	20	—	—	7	35
October 31, 1956	—	11	2	13	32	246	—	—
November 2, 1956	32	13	—	45	7	15	—	—
November 3, 1956	43	8	1	52	160	314	—	—
November 5, 1956	194	17	1	212	30	14	—	—
November 7, 1956	53	187	1	241	9	3	—	—
November 8, 1956	58	193	—	251	143	58	—	—
November 9, 1956	80	314	—	394	—	—	53	13
November 10, 1956	118	222	1	341	273	80	—	—
November 12, 1956	274	340	—	614	—	—	41	7
November 13, 1956	73	500	—	573	—	—	133	23
November 14, 1956	128	312	—	440	—	—	38	9
November 15, 1956	65	337	—	402	—	—	83	21
November 16, 1956	50	268	1	319	—	—	23	7
November 17, 1956	47	249	—	296	—	—	111	37
November 19, 1956	15	162	8	183	1	0.5	—	—
November 20, 1956	7	170	9	186	—	—	29	15
November 21, 1956	9	140	8	157	3	2	—	—
November 23, 1956	14	136	10	160	—	—	31	19
November 24, 1956	9	115	5	129	—	—	15	12
November 26, 1956	3	102	9	114	6	5	—	—
November 27, 1956	12	100	8	120	—	—	2	2

Date	No. of 1st instar	No. of large larvæ	No. of pupæ	Total	Increased by	Increased	Decreased by	Decreased
						Per cent		*Per cent*
November 28, 1956	2	100	16	118	—	—	—	—
December 26, 1956	100	308	4	412	—	—	—	—
December 27, 1956	125	284	3	412	74	18	—	—
December 28, 1956	173	310	3	486	—	—	82	17
December 29, 1956	139	262	3	404	—	—	59	15
December 31, 1956	118	223	4	345	5	1	—	—
January 2, 1957	84	263	3	350	—	—	6	2
January 3, 1957	43	300	1	344	21	6	—	—
January 4, 1957	3	360	2	365	—	—	39	13
January 5, 1957	30	276	10	316	—	—	182	58
January 7, 1957	—	133	1	134	8	6	—	—
January 8, 1957	9	130	3	142	—	—	36	25
January 9, 1957	—	105	1	106	—	—	3	3
January 10, 1957	6	97	—	103	—	—	1	1
January 11, 1957	30	71	1	102	—	—	27	26
January 12, 1957	11	63	1	75	2	3	—	—
January 16, 1957	21	56	—	77	10	13	—	—
January 28, 1957	37	50	—	87	—	—	41	47
February 4, 1957	5	41	—	46	26	57	—	—
February 6, 1957	21	51	—	72	11	15	—	—
February 7, 1957	17	66	—	83	10	12	—	—
February 8, 1957	27	66	—	93	42	45	—	—
February 9, 1957	43	92	—	135	3	2	—	—
February 11, 1957	22	116	—	138	—	—	27	19
February 12, 1957	14	97	—	111	—	—	1	.9
February 13, 1957	8	102	—	110	—	—	21	19
February 14, 1957	—	89	—	89	—	—	—	—

aqueous sugar solution or on hanging pads of honey-soaked cotton at daytime resting places and even in the absence of carabao and human baits. On the other hand, in a one-meter-cube cage in the laboratory *flavirostris* males and females would readily feed on aqueous sugar solution and would bite on man's arms.

Feeding of larvæ.—The suitability of spring water for anopheline breeding, as was demonstrated by pre-colonization test, led us to believe that supplementary food supply for larvæ was not necessary and that the continuous flow of spring water was more than enough for that purpose. The number of colony larvæ had never reached the overcrowding limit at any one time to require extra food supply. The largest larval colony population was only 614 on November 12, 1956, which was about two thirds of the 991 larvæ tested and found to be normal and healthy.

Physical characteristics of insectary stream water.—The water was inherently fresh and clear. Only during a heavy downpour would the water turn muddy and turbid but would clear up in a matter of four or six hours after the cessation of heavy rain. Water temperature ranged from 25° to 33.5°C or 77° to 92°F, taken at about high noon once a week for one year. Thompson(9) found out that the temperature normally experienced by *A. minimus* in their breeding places during the year

ranged from about 14° to 34°C, and that the full-grown *A. minimus* larvæ were kiled by a 5-minute exposure to 41°C.

Although the cage was located in an open field the insectary stream was not totally exposed to direct sunlight. The double-wall wire screen ceiling afforded a partial shade aside from the shrubs and grasses growing luxuriantly along stream banks and corners of the insectary.

The stream flowed one foot per 12 seconds at the middle but was perfectly still at the margins. The water surface was free from scum and film formations except floating grass blades and hanging plant roots. Occasional clearing of corroded material from the water filter created temporary formation of thick water-surface film and high turbidity, but in fifteen minutes the current cleared the stream of such impurities.

Chemical characteristics of insectary stream water.—The average of monthly averages of dissolved oxygen of the water during the period September 1955 to August 1956 was 4.09 ppm. (See Table 6.) De Jesus[4] found out that in *minimus*-positive places the average amount of dissolved oxygen was 5.78 ppm while in *minimus*-positive dug wells the dissolved oxygen was 2.93 ppm. In *minimus*-negative ones the gas was only 1.02 ppm. It seems that the role of dissolved oxygen in breeding water of *flavirostris* has not yet been made clear.

Our chemistry section lacked the facilities for the analysis of carbon dioxide in water, hence this is not included in our report. De Jesus (loc. cit.) seemed to imply that the lower of two concentrations of carbon dioxide is preferred by *minimus*. He concluded that temperature, turbidity, pH, iron, free and albuminoid ammonia did not affect the breeding preference of *A. minimus flavirostris*. There is no point discussing further the water analysis inasmuch as this aspect of the ecological factor of our vector species has not been well studied by workers on Philippine anophelines. The results of one-year water analysis of our insectary stream are presented in this paper (Table 6) for any future reference on the matter.

Longevity.—In our recent studies on longevity of *flavirostris* in the laboratory (unpublished report) during the dry months, from March to June, the range of survival in seven days (the usual egg maturation period) was from 1.54 to 14.28 per cent. During the rainy months of July and August the range of survival in seven days was from 20.19 to 26.27 per cent. In the

TABLE 6.—*Monthly averages of water analysis of outdoor insectary stream.*

Month	Dissolved oxygen	Hydrogen sulfide	Free chlorine	Oxygen consumed, from pot. permgt.	Free ammonia	Albuminoid ammonia	Organic nitrogen	Alkalinity	Acidity	Chlorides	Nitrite (NO_2)	Nitrate (NO_3)	Total iron	Total hardness	Manganese	Silica	Turbidity	Color	Residue on evaporation	pH	Time	Temperature
	ppm	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*			
September, 1955	5.298	0.512	0.003	1.22	0.045	0.10	0.12	223.43	38.17	6.3	0.004	0.07	0.34	171.53	0.40	35	3	4.9	—	—	11:30*	33.0°C
October, 1955	3.561	0.274	0.005	1.72	0.066	0.09	0.35	169.17	28.40	3.8	0.005	0.15	0.72	151.21	0.70	16	44	26.0	—	—	1:30**	29.9
November, 1955	4.640	0.150	0.080	1.57	0.040	0.05	0.19	187.57	40.98	6.0	0.003	0.44	0.70	186.75	0.50	26	14	19.0	472	—	11:20*	29.0
December, 1955	5.230	0.250	0.011	1.50	0.030	0.04	0.20	207.20	50.28	5.3	0.004	0.56	1.00	192.67	0.70	31	19	23.0	319	—	1:20**	28.1
January, 1956	1.810	0.300	0.009	1.80	0.028	0.08	0.25	211.90	43.83	6.2	0.022	0.06	0.50	199.29	0.28	36	15		243	—	11:10*	25.5
February, 1956	1.637	0.165	0.020	1.91	0.036	0.11	0.29	223.76	39.10	6.3	0.001	0.03	0.60	191.26	0.44	38	7	14.0	261	—	11:25*	26.4
March, 1956	2.318	0.245	0.050	2.30	0.056	0.13	0.35	226.66	46.04	7.8	0.004	0.03	1.90	215.61	0.80	32	11	26.0	239	—	10:50*	25.8
April, 1956	4.311	0.400	0.060	4.00	0.092	0.12	0.43	227.64	43.00	8.5	0.005	0.06	2.00	214.93	1.00	37	14	31.0	311	—	11:30*	29.4
May, 1956	4.065	0.416	0.030	2.80	0.480	0.28	2.30	223.79	49.54	8.6	0.002	0.06	2.70	221.01	1.37	30	21	37.0	316	—	1:30**	31.7
June, 1956	4.428	0.443	0.030	3.50	0.372	0.11	0.37	224.30	54.50	7.2	0.002	0.03	1.50	192.94	2.10	36	14	36.0	416	—	1:20**	30.2
July, 1956	4.571	0.551	0.030	1.90	0.048	0.09	0.31	217.25	44.11	8.9	0.003	0.01	1.50	206.48	2.10	39	16	35.0	295	7.2	11:30*	32.7
August, 1956	7.216	0.221	0.020	0.80	0.048	0.06	0.26	221.89	64.52	9.1	0.004	0.43	0.43	221.43	0.19	30	12	10.0	313	7.1	1:25**	31.6

* a.m.; ** p.m.

outdoor insectary, where mosquitoes were free to seek sanctuary for favorable micro-climate conditions within the cage, probably the percentage of survival was higher than in the laboratory.

Incidence of parasites and predators.—In all probability the micro-climate conditions in the outdoor insectary was not a great hindrance to the survival of mosquitoes. The high mortality rate among gravid females was due to egg parasitism. In our random observations on colony gravid females 82 per cent died with parasitised unlaid eggs; only 18 per cent had laid viable eggs. The identity of the mosquito-egg parasite still remains unknown.

As far as the colonization of *flavirostris* is concerned in an outdoor insectary the parasite hazard constitutes a major problem.

It was a common observation in outdoor insectaries that mosquito predators—such as the house lizard, jumping spiders, webbing spiders, and big red ants—would establish their respective colonies faster than the well-taken-care-of mosquitoes. Outdoor insectary protection against these highly destructive mosquito predators would require special studies. Our present inadequacies in insectary facilities and personnel would not permit us to deal with the problem of mosquito predators. Our only hope lies in an accelerated mosquito production to overcome the great losses effected by mosquito enemies.

At the rate F_1 had been produced, even at the height of *flavirostris* production (out of the average daily number of 10 gravid females, or 16.96 per cent, only two, or 18 per cent, would lay viable eggs because of egg parasitism), it would be preposterous to expect that it could establish its colony in the outdoor insectary. Unless the rate of production is raised 100 times, in order to produce 2,000 larvæ in one day or 14,000 larvæ in one week, the colonization of the species is bound to fail. In order to produce the preceding number of larvæ it would require 24,300 pupæ in one day, or 170,100 pupæ in one week, as the initial mosquito population in the insectary. These figures, estimated on daily averages, will overcome great losses from predators and egg parasites.

COLONIZATION OF A. VAGUS LIMOSUS: I. ADULTS

PROCEDURE

Gravid females were collected from harborages and carabao-baited traps and introduced as such in the insectary. The same

carabao used for feeding *flavirostris* was employed to feed *limosus*.

As *limosus* is a still-water breeder, the insectary stream was converted into a still-water canal and was polluted with carabao dung. A series of earthen breeding pots and enamel breeding pans were placed inside the insectary. Most of the breeding receptacles were filled with soil and water taken from carabao wallows, where heavy breeding of *limosus* was taking place. Other breeding containers were filled with spring water with rice straws.

RESULTS

Five days after the introduction of the first batch of gravid *limosus*, first instar larvæ appeared in all breeding waters — more in the still-water canal than in the artificial breeding pots and pans. The young larvæ appeared apparently healthy and growing faster with little mortality. As they reached the third and fourth instars they became fewer, stunted, and sluggish; and only a few reached the pupal stage. Then the pupæ died without ever developing into adults. The experiment was based on 21,683 gravid females collected during the rainy months of June, July, and August, 1957.

II. LARVÆ

PROCEDURE

In the latter part of August, when adults were becoming fewer, we turned our attention to larvæ. These were collected from carabao wallows and other breeding places and were introduced as such in the insectary still-water canal and in artificial breeding containers.

The preceding technics for larvæ were followed in the preparation of breeding receptacles, but the old soil and water were replaced with new soil and water taken from places where larvæ had been found.

RESULTS

Only fourth instar larvæ had developed into pupæ and to adults. Some of the emerged adults fed on carabao, became gravid and laid viable eggs. Some adults were weak and died without even tasting their first blood meals. The third instar larvæ had reached the fourth instar, but soon became stunted and sluggish and died before reaching the pupal stage. The first and second instar larvæ died without further development.

This experiment was based on 188,870 larvæ collected during the less rainy months of September and October, 1957. (See Table 7.)

TABLE 7.—*Total number of A. vagus limosus adults and larvæ introduced in the insectary.*

Month	No. of adults	No. of larvæ
June, 1957	12,601	——
July, 1957	5,652	——
August, 1957	16,031	——
September, 1957	——	46,260
October, 1957	——	142,610
Total	21,683	188,870

DISCUSSION

With the advent of the rainy season, it seemed easier to collect more adults in harborages and carabao-baited traps than larvæ in carabao wallows and rain pools. The presence of an enormous number of females made it expedient to obtain more larvæ from gravid females than to collect them from breeding places. We collected gravid females and introduced them as such in the insectary in the hope that in a relatively short time we could be able to populate the cage with an immense number of larvæ from which we could start our observations on larval requirements and development. In effect, the production of a large number of larvæ was immediate. The non-gravid individuals were left in their habitat until they too became gravid and ready for our immediate purpose.

When the peak of *limosus* adult production in nature had been reached and the density of imagoes began to decline with the decrease of rainfall, larvæ in carabao wallows appeared denser than adults in harborages and carabao-baited traps. To continue with the colonization of *limosus* in the belief that we might get hold of the æstivating strain of the species during the adverse dry season, we turned our attention to larvæ. Daily collections were brought to the insectary with fresh soil and water from the spot where the larvæ had been collected to simulate natural conditions. A portion of the collected larvæ was allowed to develop in the still-water canal and the rest were raised in breeding pans in the nursery for much closer observation and study.

Limosus egg layers showed little discrimination in the selection of breeding water in the insectary. They would lay their eggs on the still-water canal or in artificial water containers. However, this unselective behavior should not bt taken as habitual inasmuch as this phenomenon was only observed in a restricted space where the layers had no alternative except to dislodge themselves of eggs on available water areas.

The young larvæ hatched out from eggs in the insectary appeared healthy and grew fast with low mortality, but showed signs of arrested growth upon reaching the third and fourth instars. One could not help speculating on the possibility that there was something wrong with the breeding water. This might be due to an early exhaustion of plankton supply by the young larvæ and the slow rate of growth of larval food, which could not keep pace with the rate of consumption of the larger stages. It was, likewise, possible that the incidence of parasites and diseases had seriously affected the normal development of larvæ.

The larvæ collected from breeding places and brought to the insectary showed better results. Fourth instar larvæ had developed into pupæ and to adults. The adults fed on carabao blood, became gravid, and laid viable eggs. The third instar larvæ had reached the fourth but only to be stunted and to die without ever reaching the pupal stage. The first and second instars died prematurely without further development.

In nature the *limosus* layer would instinctively lay her eggs on undisturbed water having physical and chemical characteristics decidedly favorable for the normal development of the aquatic stages of the species. Under such a propitious situation the larvæ would mature with enough reserves of nutriments to carry on further development even in the absence, at this stage, of a rich food supply. Therefore, the removal and transfer of the fourth instar larvæ from their original habitat to a less favorable breeding medium was not a hindrance to their development to the pupal stage. Such a change in the environmental condition was perfectly tolerable to the fourth instar larvæ.

But, this was not the case with the younger larval stages. The removal of soil and water and their transfer to the insectary were unwelcome interferences in the work of nature. They brought about suppressed development. The physical and chemical characteristics of the water in its original situation could

have been seriously altered. The amount of heat the water had been receiving from sunlight each day might not be the same as what it got under the new situation. The water might have had rapid evaporation in artificial containers in the insectary to raise the chemical contents to higher concentrations. These physical and chemical changes might have or might not have directly affected the larvæ; but the sensitive micro-organisms that constituted the staple food for larvæ might not have agreed with the alteration and have ceased to exist, thereby depriving the larvæ of their means of livelihood. These drawbacks in the care of larvæ in the insectary require further studies and investigations before any attempt to colonize *limosus* should be undertaken.

OBSERVATIONS

Hackett(3) has aptly stated that "Captive insects are not normal insects," and mosquitoes are not exceptions. *A. minimus flavirostris* is well known for its ability to adapt itself to a variety of situations in nature. It can breed in flowing streams as well as in still water in dug wells; in muddy stagnant pools or in 38.5°C hot spring; and in many other unlikely places. The adults were found in humid and cool harborages along stream banks as well as in dry and warm corners of human habitations. Despite its adaptability, we could hardly find an explanation for our failure to make the species colonize itself in an outdoor insectary where all conceivable ecological conveniences were provided. Following Hackett's dictum, *flavirostris* found it unconventional to establish its colony under insectarial environment in spite of its inherent aptitude to adapt iself anywhere in nature.

Unlike *flavirostris, A. vagus limosus* is best found in carabao wallows only during the rainy season. No amount of inducement can make *limosus* live in captivity.

In the course of our experiment on colonization of *flavirostris* we also noted the following interesting phenomena:

(1) The larvæ can travel a 40-foot stretch with swift water-current through 1.5-inch diameter water pipe and come out alive at the end of the crashing trip. This was observed during the pre-colonization test on the suitability of spring water for anopheline breeding.

(2) Gravid *flavirostris* females prefer to lay eggs at the farthest end of the insectary stream and at the corner nearest to the cage wall and less at other spots at the middle of the insectary.

(3) A batch of 50 eggs laid by a single *flavirostris* female had hatched out into larvæ and developed into adults at almost the same time in a breeding pan without extra larval food supply. This particular batch of larvæ could be the offspring of a strain of *flavirostris* that could adapt itself to a variety of situations, including colonization.

(4) The author himself slept several nights inside the insectary and, in the absence of carabao bait and aqueous solution of sugar, observed the following biting behavior of *flavirostris*: (*a*) There were nights that not a single female would bite. It could suggest the existence of a strain that prefers blood other than human. (*b*) Some individuals would bite at any time of the night from dusk to dawn. Again, this could indicate that only a portion of *flavirostris* complex is anthropophilic; the greater number is zoophilic. (*c*) Two waves of 25 and 30 female *flavirostris*, respectively, had avidly bitten the author at dawn of two different nights. The tropism or stimuli that had influenced the unusual biting activities was not known. It could have been some subtle weather conditions. Frequent occurrences of unusual biting activities of the vector species could cause increase in malaria cases or result in outbreak of malaria epidemics.

SUMMARY

1. The construction of an outdoor insectary is described; the procedures and technics followed and applied in the colonization of *A. minimus flavirostris* and *A. vagus limosus* are discussed.

2. The F_1 (first filial) generation of *A. minimus flavirostris* had been consistently produced in the outdoor insectary, but owing to inadequate number of the initial adult population the first generation was not sufficient to produce the F_2.

3. The F_1 (first filial) generation of *A. vagus limosus* had also been produced in the outdoor insectary, but the larvæ of the first generation would not develop into adults owing to the seemingly unsuitable breeding water.

4. Mosquito-egg parasitism in the outdoor insectary was a great hindrance to the production of *flavirostris* eggs, considering the fact that 82 per cent of the gravid females had died with unlaid parasitised eggs and only 18 per cent had laid viable eggs.

5. Mosquito predators in the outdoor insectary constituted another major problem as they would establish their respective colonies faster than the well-taken-care-of mosquitœs.

6. Barring the preceding two major problems, it is believed the colonization of *A. minimus flavirostris* in an outdoor insectary is not a remote possibility.

7. If the number of the initial adult population introduced in the outdoor insectary were increased 100 times to overcome

the losses occasioned by enemies, *flavirostris* could have been successful in establishing its colony.

ACKNOWLEDGMENT

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BOOK REVIEWS

Books reviewed in this section represent a selection from those recieved from time to time by the National Institute of Science and Technology and the Philippine Atomic Energy Commission, two sister agencies under the National Science Development Board.

Flora fo New Zealand. Vol. 1. Indigenous Tracheophyta: Psilopsida, Lycopsida, Filicopsida, Gymnospermæ, Dicotyledons. By H. H. Allan. R. E. Owen, Government Printer, Wellington, New Zealand, 1961. liv, 1085 pp., 40 text figures. Price, £5. 5. 0.

This Flora treats of the native plants of New Zealand, a temperate country within 29° to 55° South Latitude and 158° East to 176° West Longtitude (except Lord Howe Island and Norfolk Island). The book, being a product of long experience of the author, both in the field and in the herbarium, in New Zealand vegetation, is the latest valuable treatise on the subject. The work treats of 166 families, 290 genera, 1,457 species and 280 distinct varieties. Three genera, 29 species and 61 varieties described are new to science.

Despite the untimely death of the author on October 29, 1957, when he almost completed the greater part of the manuscript, the book was finally printed in April 1961 through the efforts of Lucy B. Moore, his active associate since 1953.

The Flora includes various groups of plants with tracheid elements from the Psilopsida to Lycopsida, Filicopsida, Gymnospermæ and the Dicotyledons. Also included is a list of the Annals of Taxonomic Research on New Zealand Tracheophyta from 1769 to 1958 and the names of the authors with the country of origin or scene of work, which are excellent references. It is to be noted that the phylogenetic arrangement follows that of Dr. Holttum's classification of the ferns, which is a departure from the concept of Dr. Copeland's Genera Filicum (1947) and that of Dr. Hutchinson's The Families of Flowering Plants (1926) for the families of the Dicotyledons. The latter concept is a radical departure from that of Bentham and Hooker and that of Engler and Plantl. Hutchinson's classification, however, is based on modern practice which revolutionizes the old system.

The descriptions of the taxa are found to be so simple and

clear that a layman can follow and understand easily. The keys are dichotomous, each group is indicated by numbers. For easy reference the book treats of the description of the new taxa separately. Included in this work is a long glossary of botanical terms.

Although the Flora treated is for a temperate region yet the publication contains many plant groups which are found in the tropics like the Philippines. It is a book botanists should never miss. It shuold be a 'must" in any botanical library.—D.R.M.

Chemical Instrumentation, a Systematic Approach to Instrumental Analysis. By Howard A. Strobel. Reading, Mass. and London, Addison-Wesley Publ. Co., Inc., 1960. 653 pp. Price, $9.75.

This text is an excellent work on the basic techniques involved and the principles used in the operation of modern instruments. It is adapted to students taking an advanced course in instrumental analyses.

The reader is introduced to this new field of chemical specialization by a discussion of the science of instrumentation and errors of measurement. The text is divided into two principal parts—the opticometric method and the electrometric method of analysis. Chapters on the interaction of electromagnetic radiation and matter, and physical and geometric optics furnish the background for the discussion of the basic optical techniques presented, which are emission spectroscopy, absorption photometry, photometric instrumentation, light scattering photometry, refractometry and polarimetry. Similarly, in the second part of the text, the chapters on electrical and electro-chemical phenomena, fundamental electronics, and applications of electronics open the discussion for the consideration of the electrical methods of analysis—conductometry, potentiometry, polarography, amperometric titrations, coulometry and electrogravimetry. The last chapter deals with methods using radioisotopes.

The author's aim of conveying the importance of chemical instrumentation is well realized by a qualitative treatment of the theories and a systematic discussion of the operating procedures. Considerations for the criteria in the evaluation of the instruments as well as the requirements for their reliable operation develop the reader's perspective and ability to compare the design and performance of the different types of in-

struments. He is also presented the problems which are common in chemical research, those dealing with the methods of identification, qrantitative analysis and mensuration.

The relations between theory, design and procedure are so ably presented that the student develops a sound grasp of the basic principles involved. An extensive bibliography at the end of each chapter furnishes additional references from which he may have complete treatment of the theories, analyses or instruments discussed. The appendix gives a series of laboratory experiments to illustrate the topics covered by the various chapters.—A.M.G.

Modern University Physics. By James A. Richards, Francis Weston Sears, M. Russell Wehr and Mark W. Zemansky. Reading, Mass. and London, Addison-Wesley Publ. Co., Inc. 1960. 993 pp. Price, $7.00.

This textbook provides an introduction to classical, quantum and relativistic physics on college level. The first portion, which comprises about two thirds of the entire text, deals with classical physics. This embraces mechanics, heat, electricity and magnetism, waves and optics, which are presented in the given order.

The first chapter deals with the composition and resolution of vectors, which is the foundation of understanding the solution to physical problems. After the different branches of mechanics have been treated in the first eleven chapters, a chapter in wave motion and another in vibrating bodies are introduced. This is in order inasmuch as the notion of harmonic motion, which is dealt with in the preceding chapter, is similar to that of waves. The laws of thermodynamics, the properties of ideal gas, and the kinetic theory of gases are clearly discussed. A relatively lengthy treatment of the subject on electricity includes electric charges, potential difference, magnetic and electric fields and the properties of circuit parameters. Six chapters are devoted to the theory of propagation of light waves and their properties.

One of the noteworthy features of this book is the relatively easy-to-understand explanation of the principles involving atomic and nuclear science. A rather extensive field, this includes discussions of the quantum theory of radiation, relativity, x-rays, natural radioactivity, and nuclear energy. The chapter on nuclear energy treats of the different types of nuclear re-

actors, moderators, and neutron cross sections. Particle accelerators are also treated in the book.

The MKS system of units is generally used and to a lesser extent the English system, especially in engineering problems where this is more applicable. Units are usually included with numerics and are treated like algebraic quantities. Trigonometry and algebra are freely used throughout. More than a nodding acquaintance with elementary calculus is essential. Familiarity with chemistry, particularly on the chapters on nuclear science, is also necessary.

The student will find in this book a clear-cut and logical presentation of theory with detailed examples to clarify each principle discussed. Derivation of formulas are shown in a step-by-step, straightforward, manner. Derived formulas are boxed to put stress on their importance to the students. At the end of each chapter are problems designed to utilize the student's ability to apply the principles treated in the chapter. Answers to these problems are given at the end of the text.

Although the authors recommend this book for two-semester work, ordinary students will not find it easy to complete such an extensive subject-matter within that period. The book is, nevertheless, fully recommended for students of science and engineering.—G.R.J.

Calculus and Analytic Geometry. By George B. Thomas, Jr. Reading, Mass. and London, Addison-Wesley Publ. Co., Inc. 1960. 1010 pp. Price, $9.75.

This textbook is intended for students of science and engineering who have some familiarity with algebra and trigonometry. The first few chapters introduce the concepts of limits and derivative of functions. The introduction of integral calculus here at a very early stage (in chapters 4 and 5) is not usual with texts of calculus. A brief review of trigonometry is also included.

An interesting feature is the treatment of analytic geometry, which is not treated as a separate subject but as a tool of calculus and is introduced wherever its necessity warrants. This makes the students comprehend more easily the interrelation of analytic geometry with calculus. Determinants, which are normally a part of algebra, are also briefly discussed. Examples of applications are freely demonstrated throughout. They

give the students a better appreciation of the subject as a powerful tool in applied fields. The last portion of the text is devoted to the treatment of infinite series, complex numbers and elementary differential equations.

Another noteworthy feature of the book is the simple, clear and accurate presentation of definitions, theorems and general procedures. Scattered throughout are practice problems which, no doubt, will enhance the students' ability for better logical reasoning. Answers to these problems are given at the end of the text for reference.

Designed for three-semester work, the book, in general, is highly recommended for students of mathematics and engineering.—G.R.J.

Plant Analysis and Fertilizer Problems. Edited by Walter Reuther. Washington 6, D. C., American Institute of Biological Sciences, 1960. 454 pp. Price, $8.00.

This useful collection of scientific papers prepared by different researchers from different countries is intended for the use of fruit growers and agricultural specialists who desire to increase the food-production potential of their orchards, gardens and farms through the application of modern scientific principles of soil fertility and plant nutrition.

The chapters in Part I describe methods by which chemical leaf analysis may be used to detect and determine the needed elements of fruit trees and annual crops. Foliar analysis may also be used to determine color development in certain fruit trees. In cases where neither leaf analysis nor visual symptoms suffice to confirm a diagnosis and to determine nutritional deficiencies, soil analysis can be of some help. The relation between the concentration of nutrients in plants and the so-called critical level has been determined for several crops with the use of especially designed statistical formulae.

Part II deals with basic studies on plant composition in connection with foliar-applied nutrients and ion interactions in diagnostic work of plant tissues. The second section takes care of some influences of mineral nutrition on organic nutrition in studies made in the U. S. A., Israel, and Japan. The third section stresses the fact that other factors, like environment, growth, and disorders, also affect plant composition by either enhancing or depressing certain nutrient elements.

Part III gives a short discussion of the shortcomings that were encountered in the studies that compose Parts I and II. It includes also the improvements that may be made upon these studies so that plant-tissue analysis may become a useful and powerful research and diagnostic tool in the solution of present fertilizer problems.—J.R.S.M.

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[New names and new combinations are printed in italics.]

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